Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series

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Therapy-related myelodysplasia and myeloid leukemia (t-MDS/t-AML) is a distinctive clinical syndrome occurring after exposure to chemotherapy (CT) or radiotherapy (RT). We report findings on 306 consecutive patients referred to our institution with morphologic review and cytogenetic analyses. Since 1972, 141 males and 165 females with a median age of 51 years (range, 3-83 years) at primary diagnosis and 58 years (range, 6-86 years) at secondary diagnosis were analyzed. Patients had been administered various cytotoxic agents, including alkylating agents (240 patients, 78%) and topoisomerase 2 inhibitors (115 patients, 39%). One hundred twenty-one (40%) had undergone CT alone, 43 (14%) had undergone RT alone, and 139 (45%) had undergone both modalities. At diagnosis of t-MDS/t-AML, 282 (92%) had clonal abnormalities involving chromosome 5 (n = 63), chromosome 7 (n = 85), chromosomes 5 and 7 (n = 66), recurring balanced rearrangements (n = 31), other clonal abnormalities (n = 39), or normal karyotype (n = 24). Abnormalities of chromosome 5, 7, or both accounted for 76% of all cases with an abnormal karyotype. Seventeen patients acquired t-MDS/t-AML after autologous stem cell transplantation, but no unique pattern of cytogenetic abnormalities was observed. Shorter latency was observed for patients with balanced rearrangements (median, 28 vs 67 months; *P* < .0001). Patients with acute leukemia were more likely to have balanced rearrangement than those with myelodysplasia (28% vs 4%; *P* < .0001). Median survival time after diagnosis of t-MDS/t-AML was 8 months; survival at 5 years was less than 10%. These data confirm and extend previous associations between clinical, morphologic, and cytogenetic findings in t-MDS/t-AML. (Blood. 2003;102:43-52)

Introduction

Therapy-related myelodysplasia and myeloid leukemia (t-MDS/t-AML) is a well-recognized clinical syndrome occurring as a late complication after cytotoxic therapy.[1-12] The latency period between primary diagnosis and therapy-related disease ranges between several months to several years, and it may be dependent on the cumulative dose or dose intensity of the preceding cytotoxic therapy and on the exposure to specific agents. The clinical course is typically progressive and relatively resistant to conventional therapies used for leukemias arising de novo.[2,7,9,12] A spectrum of morphologic abnormalities is observed, with a continuum in the percentage of marrow blasts often used to describe therapy-related myelodysplastic syndrome (t-MDS) and therapy-related acute myeloid leukemia (t-AML). Older classification schemes have used the criterion of 30% blasts to distinguish the 2 disorders, whereas the recent World Health Organization (WHO) classification groups them together, emphasizing their common features.[13] Regardless of the initial percentage of blasts, t-MDS and t-AML represent a single distinctive syndrome.

Most patients with t-MDS/t-AML have clonal chromosomal abnormalities in their bone marrow cells at diagnosis. We previously reported that 61 of 63 (97%) t-MDS/t-AML patients had a clonal abnormality and that in 55 (87%) there was loss of all or part of chromosome 5 or 7 or both.[1,2,10] Many others have since confirmed this finding.[4,5,7,9] More recently, we and others observed that a distinct subset of t-MDS/t-AML patients have balanced translocations often involving chromosome bands 11q23 or 21q22 and the *MLL* or *RUNX1/AML1* genes, respectively.[14-23] In addition, balanced rearrangements characteristic of certain leukemias commonly presenting de novo, such as t(15;17) in acute promyelocytic leukemia and inv(16)(q13q22) in M4Eo, have been observed after cytotoxic therapy.[8,10,24]

In this report, we expand on our previously published cases with new clinical and cytogenetic data on 306 patients with t-MDS/t-AML evaluated at the University of Chicago.[10,25,26] The data have been used to confirm previous findings and to investigate other pertinent issues, including disease latency, clinical correlation with cytogenetic abnormalities, and features related to survival. Specific analyses of our database address the following questions: (1) Is there a correlation between primary treatment modality (ie, chemotherapy or radiotherapy) and the development of specific subtypes of t-MDS/t-AML? (2) What is the latency interval from primary therapy to the first evidence of bone marrow dysfunction? (3) Is the high frequency of abnormalities of chromosomes 5 and 7 confirmed in this expanded series, and, if so, how are these abnormalities related to the development of t-MDS/t-AML? (4) Is there a relationship between the chromosomal changes and either the

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primary disease or the type of primary therapy (exposure to specific agents)? (5) Is the clinical course of t-MDS/t-AML with balanced translocations of 11q23 or 21q22 different from that seen in patients with abnormalities of chromosomes 5, 7, or both? (6) Do distinctive cytogenetic features to t-MDS/t-AML occur after high-dose chemoradiotherapy and autologous stem cell transplantation? (7) Are specific chromosomal abnormalities associated with better or worse survival after the diagnosis of t-MDS/t-AML?

Patients, materials, and methods

Case definitions

Between 1972 and July 2001, after clinical and morphologic review in our laboratories, 306 consecutive patients referred to the University of Chicago were confirmed to have a diagnosis of t-MDS/t-AML. All patients had undergone chemotherapy (CT), radiation therapy (RT), or a combination of them for an antecedent disorder. Approximately half the patients had been treated for their primary disease at our institution. However, the total number of similarly treated patients at risk for t-MDS/t-AML is not available; therefore, incidence rates and relative risks cannot be determined from these data. Many patients were referred to the University of Chicago from local institutions for evaluation and treatment only after the development of t-MDS/t-AML or after clinical information and bone marrow slides on patients were forwarded when a specimen was sent to our institution for cytogenetic analysis. Approval was obtained from the University of Chicago institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki.

Clinical data were collected, and details concerning the primary treatment were obtained from a review of each patient's medical history. For referral cases, clinical data were gathered by direct communication with the referring physician. RT ports, the dose of each treatment course in grays, and the doses and duration of each chemotherapy course were determined whenever possible for each patient. In some instances, totals reported in the following tables do not equal 306 because of missing data. Patients were followed up until death or through June 2002, except for 17 (6%) patients who were lost to follow-up. The duration of a myelodysplastic phase, if any, and the length of survival from the time of initial bone marrow dysfunction were noted. Latency interval was defined as starting with the first cytotoxic therapy and ending with the first bone marrow examination showing therapy-related MDS or myeloid leukemia. Treatments given to patients after the development of t-MDS/t-AML were individualized and variable, ranging from supportive care only to intensive chemotherapy to bone marrow transplantation. Thus, response data are not reported here.

Chemotherapy agents were classified by mechanism of action. Alkylating agents include melphalan, cyclophosphamide, ifosfamide, nitrogen mustard, chlorambucil, busulfan, dacarbazine (DTIC), procarbazine, carmustine (BCNU), lomustine (CCNU), mitomycin C, PCNU, semustine (methyl-CCNU), carboplatin, cisplatin, thiotepa, and hexamethylmelamine. Topoisomerase 2 inhibitors include etoposide (VP16), teniposide (VM26), doxorubicin, daunorubicin, mitoxantrone, and amsacrine. Antimetabolites include fluorouracil (5FU), fluorodeoxyuridine (FUDR), methotrexate, cytarabine, 6-mercaptopurine, 6-thioguanine, hydroxyurea, azathioprine, cladribine, fludarabine, and pentostatin. Only 15 patients had received 1 of these last 3 drugs (purine analogs), and all of them had also received alkylating agents; 9 had received several other agents as well. Antitubulin agents include vincristine, vinblastine, vindesine, paclitaxel, and docetaxel.

The diagnosis of t-MDS was made when the patient's peripheral blood and bone marrow cells showed features of dyspoiesis, as defined by the French-American-British (FAB) criteria for MDS and as described by us and others[1-3,5-7] as characteristic of the changes seen in t-MDS. Because most cases in the current series occurred before the new WHO classification, the FAB criteria were used. Patients were classified as having t-MDS if the percentage of blasts in the marrow was less than 30%, whereas overt t-AML was diagnosed if the percentage of blasts was 30% or higher, as determined from marrow aspirates or as judged from marrow biopsy sections when increased reticulin prevented aspiration. As noted in other studies, a substantial number of cases could not be readily classified or were atypical according to FAB criteria. Among patients for whom follow-up was available, we recorded the evolution from t-MDS to t-AML using the threshold criterion of 30% or more bone marrow blasts.

Cytogenetic analysis

Cytogenetic analyses were performed with quinacrine fluorescence and trypsin-Giemsa banding techniques on bone marrow cells from aspirates or biopsy specimens and on peripheral blood cells obtained at the time of diagnosis. Metaphase cells examined were obtained from direct preparations and from 24- or 48-hour unstimulated cultures. Chromosomal abnormalities are described according to the International System for Human Cytogenetic Nomenclature.[27]

Statistical methods

Associations between categorical variables were analyzed using χ^2 analysis or Fisher exact test. Categories were sometimes pooled to address specific questions as noted. In some instances, totals reported in the tables in "Results" do not equal 306 because of missing data. Percentages have been rounded to the nearest whole number. A logistic regression model was fit to examine the relationship between age at primary diagnosis and the occurrence of specific chromosomal abnormalities. Kruskal-Wallis nonparametric tests[28] were used to compare latency intervals between groups. Survival rates were estimated by the Kaplan-Meier method[29] and compared between groups using the log-rank test.[30] *P* values for overall tests are shown in the tables, and values of .05 or less are regarded as statistically significant. Additional *P* values are provided in the text to facilitate subgroup comparisons and to allow for multiple testing. Only *P* values less than or equal to .01 are regarded as statistically significant; values between .01 and .05 are considered marginally significant.

Results

Clinical characteristics

Clinical characteristics of the 306 patients with t-MDS/t-AML are shown in Table 1. One hundred seventy-one (56%) patients had primary hematologic malignancy, and nearly equal numbers of patients had Hodgkin disease (HD; 77 patients [25%]) and non-Hodgkin lymphoma (NHL; 70 patients [23%]). One hundred seventeen (38%) patients had a solid tumor as the primary

Table 1. Primary diagnoses and primary cytotoxic therapy received by 306 patients in whom t-MDS/t-AML developed

Primary diagnosis	No. patients	CT only (%)	RT only (%)	CMT (%)
No malignancy	18	12 (67)	2 (11)	4 (22)
Hematologic malignancy	171	69 (40)	5 (3)	97 (57)
Hodgkin disease	77	18 (23)	4 (5)	55 (71)
Non-Hodgkin lymphoma	70	33 (47)	1 (1)	36 (51)
Myeloma	23	17 (74)	0	6 (26)
Other*	1	1 (100)	0	0
Solid tumor	117†	40 (35)	36 (32)	38 (33)
Breast	32‡	11 (35)	5 (16)	15 (48)
Ovary	15	12 (80)	1 (7)	2 (13)
Prostate	13§	0	11 (100)	0
Lung	9	5 (56)	2 (22)	2 (22)
Cervix	7	0	4 (57)	3 (43)
Other*	41	12 (29)	13 (32)	16 (39)
Totals	306†	121 (40)	43 (14)	139 (46)

*Smaller diagnostic groups were not further subdivided by primary diagnosis.
†In 3 patients, the primary therapy was incompletely known.
‡In 1 patient, the primary therapy was incompletely known.
§In 2 patients, the primary therapy was incompletely known.

malignancy. Breast cancer was the most common among these (32 patients [10%]). Importantly, we also studied 18 (6%) patients who had not had a prior malignancy. However, these patients had undergone cytotoxic therapy for the treatment of autoimmune disorders (2 with rheumatoid arthritis, 2 with Behçet syndrome, 2 with polymyositis, 1 with immune thrombocytopenia), immunosuppression for renal allografts (n = 5), hydatidiform mole (n = 1), and hepatitis (n = 1). The specific indication for cytotoxic therapy was not known for 4 patients who had no prior diagnosis of malignancy.

The series includes 165 women and 141 men with the following racial/ethnic distribution: 199 white (82%), 40 African American (16%), 5 Hispanic (2%), and 62 with unrecorded race/ethnicity. Among 299 patients whose age at the time of initial primary disease diagnosis was known, the median age was 51 years (range, 3-83 years).

Table 1 also lists whether the patients' primary cytotoxic therapy consisted of CT, RT, or combined modality therapy (CMT). One hundred twenty-one (40%) patients had only CT, and 43 (14%) had only RT. Usually, the RT-only patients had received radiation to large ports encompassing areas of active hematopoiesis within the central skeleton and pelvis. One hundred thirty-nine (46%) patients had been treated with both modalities, either concurrently as part of the initial treatment plan (54 patients) or sequentially, often separated by several years, for treatment of relapsed disease (53 patients). For 32 of the CMT patients, the treatment records were incomplete with regard to concurrent or sequential therapy.

Further analysis of these treatment groups according to clinical presentation and later progression of t-MDS is shown in Table 2. Overall, 224 (73%) patients were first diagnosed in the t-MDS phase of disease; progression to t-AML was later observed in 98 patients, but follow-up bone marrow data were incomplete in another 54 (18%) patients. The median time for progression from t-MDS to t-AML was 4 months; the interquartile range (IQR; 25th-75th percentiles) was 2.0 to 8.0 months. Eighty-two (27%) patients had overt t-AML on presentation. Of the 121 patients who underwent only CT, 93 (77%) had t-MDS on presentation; t-MDS progressed to t-AML in 36 patients, no change occurred before death in 26 patients, and follow-up hematology data were incomplete in 31 patients. Twenty-eight (23%) of the CT-only patients had overt t-AML on presentation. Of the 43 patients who underwent only RT, 28 (65%) patients had t-MDS and 15 (35%) had t-AML on presentation. Among the remaining 139 patients who had undergone CT and RT, 103 (74%) patients had t-MDS and 36 (26%) had t-AML on presentation. In 51 of the 103 patients, t-MDS progressed to t-AML; 33 had t-MDS only, and for 19 patients follow-up hematology data were incomplete. The type of primary diagnosis (no malignancy, hematologic malignancy, or solid tumor) was associated with the clinical presentation ($P = .011$). In a pairwise comparison, patients with solid tumors (38%) were more likely to have overt t-AML on presentation than patients with hematologic malignancy (19%; $P < .001$) or with nonmalignant disorders (22%; $P = .29$).

In Table 3, latency intervals for the development of t-MDS/t-AML are shown according to patients' primary diagnosis and primary therapy and also according to age at primary diagnosis and clinical presentation. Median latency overall was 62 months (IQR, 35-107 months), but this varied from 28 to 136 months for different subgroups. Patients with nonmalignant primary diagnoses had longer latency intervals ($P = .01$). Younger patients also tended to have longer latency intervals ($P < .0001$), and this association remained when the 18 patients with nonmalignant conditions were excluded ($P < .0001$). However, this apparent association could be attributed in part to competing risks as older patients died of other age-related factors before the development of t-MDS/t-AML.

As shown in Table 3, the latency period for the development of t-MDS did not differ between those who had only t-MDS (median, 58 months), those with t-MDS that later evolved to t-AML (median, 65 months), and those with overt t-AML on presentation (median, 54 months) ($P = .15$). However, the median latency interval was 65 months for the 220 t-MDS patients for whom information regarding latency is known, which was longer than the 54 months for the 74 patients with t-AML on presentation ($P = .04$). The IQR for the latency for patients with t-AML on presentation was also narrower (range, 28-88 months) than for the patients with t-MDS (range, 39-110 months) on presentation.

Table 2. Clinical presentation by primary diagnosis and primary therapy

Clinical feature	No. patients	t-MDS→unknown (%)	t-MDS (%)	t-MDS→t-AML (%)	t-AML (%)
Primary diagnosis*					
No malignancy	18	6 (33)	3 (17)	5 (28)	4 (22)
Hematologic malignancy	171	33 (19)	44 (26)	61 (36)	33 (19)
Hodgkin disease	77	14 (18)	13 (17)	33 (43)	17 (22)
Non-Hodgkin lymphoma	70	12 (17)	26 (37)	22 (31)	10 (14)
Myeloma	23	7 (30)	5 (21)	6 (26)	5 (22)
Other	1	0	0	0	1 (100)
Solid tumor	117	15 (13)	25 (21)	32 (27)	45 (38)
Breast	32	4 (13)	2 (6)	14 (44)	12 (38)
Ovary	15	1 (7)	2 (13)	3 (20)	9 (60)
Prostate	13	2 (15)	5 (38)	0	6 (46)
Lung	9	0	2 (22)	2 (22)	5 (56)
Cervix	7	1 (14)	3 (43)	1 (14)	2 (29)
Other	41	7 (17)	11 (27)	12 (29)	11 (27)
Totals	306	54 (18)	72 (24)	98 (32)	82 (27)
Primary therapy†					
CT only	121	31 (26)	26 (21)	36 (30)	28 (23)
RT only	43	4 (9)	13 (30)	11 (26)	15 (35)
CMT	139	19 (14)	33 (24)	51 (37)	36 (26)

t-MDS→unknown indicates patients with t-MDS for whom no further follow-up data were available regarding progression to t-AML before death.
*Fisher exact test for the major categories of no malignancy, hematologic malignancy, and solid tumor (3 × 4 contingency table); $P = .011$.
†$P = .07$.

Table 3. Clinical features and latency intervals from first cytotoxic therapy to presentation with t-MDS/t-AML

Clinical feature	No. patients	Latency, mo		P
		Median	IQR	
Primary diagnosis				.01*
No malignancy	18	130	54-228	—
Hematologic malignancy	167	64	40-97	—
Hodgkin disease	75	62	42-85	—
Non-Hodgkin lymphoma	69	68	40-115	—
Myeloma	22	52	34-72	—
Other	1	122	—	—
Solid tumor	109	55	25-105	—
Breast	30	65	25-107	—
Ovary	12	53	18-74	—
Prostate	12	54	18-82	—
Lung	9	28	19-43	—
Cervix	7	136	79-396	—
Other	39	52	33-109	—
Primary therapy				.19
CT only	116	53	33-106	—
RT only	42	68	25-135	—
CMT	135	67	45-104	—
Age range at primary diagnosis, y				< .0001
3-36	73	72	46-134	—
37-50	73	82	48-151	—
51-61	70	54	28-77	—
62-83	78	47	24-82	—
Presentation of t-MDS/t-AML				.04†
t-MDS	220‡	65	39-110	—
t-MDS→unknown	53	73	44-115	—
t-MDS only	71	58	35-96	—
t-MDS→t-AML	96	65	36-115	—
t-AML	74	54	28-88	—

— indicates not applicable; t-MDS→unknown, patients with t-MDS for whom no further follow-up data were available regarding progression to t-AML before death.

*For comparison of the 3 major categories of no malignancy, hematologic malignancy, and solid tumor.

†For pairwise comparison of t-MDS and t-AML.

‡Data regarding latency interval were missing for 4 patients with t-MDS.

Cytogenetic analysis

Table 4 summarizes the clonal cytogenetic abnormalities observed in the 306 patients with t-MDS/t-AML. Some of these karyotypes have been published in detail.[1-3,6,8,10,18,20,22,25,30] Twenty-four (8%) patients had no detectable abnormality, and 282 (92%) patients had one or more detectable abnormal clones. The most common abnormalities involved loss of a whole chromosome 5, 7, or both (−5, −7) or a deletion of the long arm of these chromosomes [del(5q) or del(7q)] in 214 (70%) patients. Monosomy 7 (−7) was present in 102 (33%) patients and monosomy 5 (−5) in 36 (12%) patients. The most common structural chromosomal abnormality was a del(5q) in 59 (19%) patients.

Analysis of the clonal cytogenetic abnormalities based on the primary diagnosis and the primary therapy is shown in Table 5. There was a significant difference among the 3 primary diagnosis categories in the fraction of patients with abnormalities of chromosomes 5 and 7 (83%, 75%, and 59% for no malignancy, hematologic malignancy, and solid tumor, respectively; $P = .007$). Clonal abnormalities of chromosome 5, 7 or both were most common among patients with multiple myeloma (83%) and nonmalignant primary disorders (83%) and less common, though still frequent, among patients with solid tumors (59%). Abnormalities of chromosome 5, 7 or both were found in 19 (59%), 9 (60%), and 5 (38%) patients with breast, ovarian, and prostate carcinoma, respectively. In contrast, three fourths of patients with malignant lymphoma (HD, 73%; NHL, 76%) had an abnormality of chromosome 5 or 7.

Similar analysis based on the primary treatment modality revealed a clonal abnormality of chromosome 5, 7, or both in 84 (69%) patients treated with CT only, in 26 (60%) patients with prior RT alone, and in 100 (72%) patients with prior CMT; these differences were not statistically significant ($P = .36$).

Balanced translocations involving bands 11q23 and 21q22 occurred in 10 (3%) and 8 (3%) patients, respectively; t(15;17) was observed in 6 (2%) patients, and inv(16) occurred in 6 (2%) patients. Recurring balanced rearrangements were seen in 9 (5%) patients with a hematologic malignancy, 21 (18%) with a solid tumor, and in 1 of the 18 patients with a nonmalignant disorder. One male who underwent MOPP/ABVD for 6 cycles for Hodgkin disease had 2 recurring cytogenetic abnormalities, del(5q) and t(3;21), in the same clone. One patient with colon cancer acquired t-MDS/t-AML with inv(16)(p13q22) and del(7q) after CMT. One patient with lung cancer patient who underwent only RT had t(15;17) and trisomy 8. Eleven patients with balanced rearrangements were identified among patients treated with CT alone, 6 patients with RT alone, and 14 patients with CMT.

Correlations between clonal cytogenetic abnormalities and clinical presentation of therapy-related disease and age at primary diagnosis are shown in Tables 6 and 7. Abnormalities of chromosomes 5, 7, or both were observed in 172 of 224 (77%) patients with t-MDS on presentation compared with 42 of 82 (51%) with overt t-AML on presentation. Balanced rearrangements occurred more frequently in the subgroup with t-AML than in those with t-MDS (28% vs 4%; $P < .0001$) (Table 6). Normal karyotypes were observed in 19 (8%) patients with t-MDS and in 5 (6%) patients with t-AML. There was no statistically significant association between age at primary diagnosis and cytogenetic subgroup (Table 7). Treating age at primary diagnosis as a continuous variable in a logistic regression model, the relative frequency of balanced rearrangements or of other clonal abnormalities not involving chromosomes 5 or 7 was not associated with age ($P = .65$).

Latency periods for the development of bone marrow dysfunction from the time of first cytotoxic therapy and according to cytogenetic features are presented in Table 8. Patients with balanced rearrangement had shorter latency intervals than all other patients taken together (median, 28 vs 67 months; $P < .0001$).

Table 4. Cytogenetic abnormalities in 306 patients with t-MDS/t-AML

Karyotype	No. (%)
Normal karyotype	24 (8)
Clonal abnormalities	282 (92)
Clonal abnormalities of chromosome 5, 7, or both (± other abnormalities)	214 (70)
Abnormal chromosome 5*	63 (21)
Abnormal chromosome 7*	85 (28)
Abnormal chromosomes 5 and 7	66 (22)
Recurring balanced rearrangements	31 (10)
t(11q23)	10 (3.3)
t(21q22)*	8 (2.6)
t(15;17)	6 (2.0)
inv(16)*	6 (2.0)
t(8;16)	1 (0.3)
Other clonal abnormalities	39 (13)†

*One patient with an abnormality of chromosome 5 and t(3;21) and one patient with an abnormality of chromosome 7 and inv(16) are listed twice in the table.

†Includes eight patients with +8, 3 patients with −13/del(13q), and 1 patient each with del(20q), del(11q), +11, +21, or −Y.

Table 5. Primary diagnosis, primary therapy, and clonal cytogenetic abnormalities in 306 patients with t-MDS/t-AML

Clinical feature	No. patients	Abnormality 5 (%)	Abnormality 7 (%)	Abnormalities 5 and 7 (%)	Balanced rearrangement (%)	Other abnormalities (%)	Normal (%)
Primary diagnosis*							
No malignancy	18	1 (6)	13 (72)	1 (6)	1 (6)	1 (6)	1 (6)
Hematologic malignancy	171	32 (19)	49 (29)	47 (27)	9 (5)	25 (15)	9 (5)
Hodgkin disease	77	13 (17)	26 (34)	17 (22)	4 (5)	14 (18)	3 (4)
Non-Hodgkin lymphoma	70	12 (17)	15 (21)	26 (37)	4 (6)	9 (13)	4 (6)
Myeloma	23	7 (30)	8 (35)	4 (17)	0	2 (9)	2 (9)
Other	1	0	0	0	1 (100)	0	0
Solid tumor	117	29 (25)	22 (19)	18 (15)	21 (18)	13 (11)	14 (12)
Breast	32	6 (19)	10 (31)	3 (9)	8 (25)	3 (9)	2 (6)
Ovary	15	5 (33)	1 (7)	3 (20)	3 (20)	3 (20)	0
Prostate	13	3 (23)	0	2 (15)	1 (8)	4 (31)	3 (23)
Lung	9	1 (11)	2 (22)	1 (11)	4 (44)	1 (11)	0
Cervix	7	2 (29)	2 (29)	1 (14)	0	0	2 (29)
Other	41	12 (29)	7 (17)	8 (20)	5 (12)	2 (5)	7 (17)
Primary therapy†							
CT only	121	21 (17)	38 (31)	25 (21)	11 (9)	15 (12)	11 (9)
RT only	43	14 (33)	6 (14)	6 (14)	6 (14)	5 (12)	6 (14)
CMT	139	26 (19)	40 (29)	34 (24)	14 (10)	18 (13)	7 (5)

Two patients, one each with abnormal chromosome 5 or 7, are counted only in the balanced rearrangement category because of t(3;21) or inv(16). Patients with abnormalities of 5, 7, or both plus other abnormalities except balanced rearrangement are counted only in the abnormal chromosome 5, 7, or both categories.

*$P < .0001$ as measured by Fisher exact test for comparison of the 3 major categories of no malignancy, hematologic malignancy, and solid tumor (3×6 contingency table).

†$P = .19$.

Table 9 shows the association between specific cytogenetic subgroups and prior exposure to various CT drugs or RT for the patients for whom these data were available. Few patients received single-agent treatment, and most patients in this series received more than one class of CT. Thus, conclusions could not be made regarding specific types of CT and associated cytogenetic abnormalities. However, patients who received topoisomerase 2 inhibitors were more likely to have a balanced rearrangement or another clonal abnormality (not involving chromosome 5 or 7) than patients who did not receive these agents (32% vs 16%; $P = .002$). Table 10 shows latency periods according to classes of CT agents or RT. The observation of similar latency periods for patients treated with different classes of CT agents may be related to the fact that 82% of all patients in this series received an alkylating agent. Only 3 patients received topoisomerase 2 inhibitors without exposure to alkylating agents, precluding a statistically meaningful comparison of latency intervals. Median latency periods (53-67 months) for each treatment subgroup are consistent with those observed after alkylator exposure. The median latency period of patients receiving CMT was 67 months, providing little evidence for potentiation of the leukemogenic effect of RT on CT alone.

t-MDS/t-AML after autologous stem cell transplantation

Within this series of 306 patients, 17 patients (10 male, 7 female) acquired t-MDS/t-AML after high-dose therapy and autologous hematopoietic stem cell transplantation (SCT). The preparative regimen included total body irradiation (TBI) in 2 patients. The source of stem cells was bone marrow in 3 patients, peripheral blood in 10 patients, peripheral blood and bone marrow in 1 patient, and unknown in 3 patients. Primary diagnoses included HD (n = 9), NHL (n = 7), and breast cancer (n = 1). The HD patients included 8 with t-MDS (with one progressing to t-AML) and 1 with overt t-AML. The frequency and distribution of cytogenetic abnormalities in the patients with t-MDS/t-AML after SCT were similar to those of the entire group of t-MDS/t-AML, and no abnormalities were unique to patients with t-MDS/t-AML after SCT. Cytogenetic abnormalities in the HD patients included abnormal chromosome 5 or 7 (n = 5), monosomy 13 (n = 1), t(21q22) (n = 1), and other abnormalities (n = 2). Of the NHL patients, 5 had t-MDS and 2 had t-AML. Cytogenetic abnormalities included abnormal chromosome 5, 7, or both (n = 5), t(15;17) (n = 1), and t(21q22) (n = 1). The patient with breast cancer had overt t-AML on presentation and an abnormality of chromosome 5 and trisomy 8. The median time to t-MDS/t-AML was only 22 months after SCT. However, the latency period from first cytotoxic therapy to t-MDS/t-AML was similar for the 16 patients with HD or NHL who underwent SCT and the 131 patients with HD or NHL who did not undergo SCT (median, 66 vs 65 months; $P = .87$) (Figure 1).

Survival

Only 26 patients are known to be alive; survival status is unknown for 17 (6%) patients who were lost to follow-up. The median time

Table 6. Clinical presentation and clonal cytogenetic abnormalities in patients with t-MDS/t-AML

Presentation	No. patients	Abnormality 5 (%)	Abnormality 7 (%)	Abnormalities 5 and 7 (%)	Balanced rearrangement (%)	Other abnormalities (%)	Normal (%)
t-MDS→unknown	54	10 (19)	20 (37)	9 (17)	2 (4)	7 (13)	6 (11)
t-MDS only	72	19 (26)	16 (22)	17 (24)	0	11 (16)	9 (13)
t-MDS→t-AML	98	19 (19)	32 (33)	28 (29)	6 (6)	9 (9)	4 (4)
t-AML only	82	14 (17)	16 (20)	12 (15)	23 (28)	12 (15)	5 (6)
Totals	306	62 (20)	84 (27)	66 (22)	31 (10)	39 (13)	24 (8)

$P < .0001$. Two patients, one each with abnormal chromosome 5 or 7, are counted only in the balanced rearrangement category because of t(3;21) or inv(16).

Table 7. Age at primary diagnosis and clonal cytogenetic abnormalities in t-MDS/t-AML

Age range in quartiles, y	No. patients	Abnormality 5 (%)	Abnormality 7 (%)	Abnormalities 5 and 7 (%)	Balanced rearrangement* (%)	Other abnormalities (%)	Normal (%)
3-36	73	11 (15)	21 (29)	14 (19)	7 (10)	12 (16)	8 (11)
37-50	76	17 (22)	28 (37)	16 (21)	9 (12)	4 (5)	2 (3)
51-61	70	15 (21)	19 (27)	14 (20)	8 (11)	10 (14)	4 (6)
62-83	80	18 (23)	16 (20)	19 (24)	7 (9)	10 (13)	10 (13)
Totals	299	61 (20)	84 (28)	63 (21)	31 (10)	36 (12)	24 (8)

$P = .34$.

*Two patients, one each with abnormal chromosome 5 or 7, are counted only in the balanced rearrangement category because of t(3;21) or inv(16).

from diagnosis of t-MDS/t-AML to death was 8 months (95% confidence interval [CI], 7-9 months) (Figure 2). The median survival of patients with t-MDS at presentation was 8.6 months (95% CI, 7.6-9.9 months) compared with 6.9 months (95% CI, 4.0-8.5 months) for patients with t-AML on presentation. Twenty-five percent of patients with overt leukemia died within 1 month of diagnosis compared with only 3% of patients with t-MDS. Kaplan-Meier survival curves are shown in Figure 3 for each major cytogenetic subgroup. Median survival times after the diagnosis of t-MDS/t-AML by cytogenetic group were 7 months for chromosome 5 abnormalities, 9 months for chromosome 7 abnormalities, 5 months for combined chromosome 5 and 7 abnormalities, 11 months for recurring balanced rearrangements, 9 months for other clonal abnormalities, and 11 months for normal karyotypes (log-rank, $P = .017$). Patients with abnormalities of chromosomes 5 and 7 had the worst overall survival compared with all other groups (log-rank, $P = .005$).

Discussion

Our analysis of 306 patients represents the largest single-institution series of t-MDS/t-AML reported. The data presented extend our earlier findings that t-MDS/t-AML occurring after CT with alkylating agents, radiation, or both is a distinctive hematopoietic disorder usually characterized by the presence of losses and deletions of chromosomes 5 and 7. Therapy-related leukemia develops in patients undergoing CT or RT, alone or in combination, and the results are clinically and cytogenetically similar. The median latency interval from primary therapy to the first evidence of bone marrow dysfunction was 64 months for patients with primary hematologic malignancies and 55 months for those with primary solid tumor malignancies. Latency was noticeably longer (130 months) among the smaller number of patients treated for prior nonmalignant conditions. Our data do not demonstrate an association between the modality of primary therapy (CT or RT) and the latency between initial treatment and the onset of bone marrow dysfunction. Similarly, we did not identify any relationship between chromosomal changes and the primary treatment modality (Table 5). With the exception of topoisomerase 2 inhibitors, there is no detectable association between specific chromosomal changes and the class of chemotherapeutic agent used. This latter analysis is made difficult, however, by the polychemotherapy that many patients received. Specific information on dose, timing, and duration of alkylating agent therapy was not available or was heterogeneous. Recurring balanced rearrangements were significantly more common in patients who had previously undergone therapy for a solid tumor and in those who had received a topoisomerase 2 inhibitor. These patients were also significantly more likely to have overt t-AML and short latency periods. Median survival after the diagnosis of t-MDS/t-AML was only 8 months.

Poor survival of patients with t-MDS/t-AML is a function of multiple competing risks, including persistence of the primary malignant disease, significant organ dysfunction from prior therapies, prolonged immunocompromised status, and lack of uniform treatment. Most of our patients had abnormalities of chromosomes 5, 7, or both, abnormalities that portend poor survival when observed in patients with AML de novo. Median survival times of

Table 8. Latency intervals according to primary diagnosis and cytogenetic features in patients with t-MDS/t-AML

Primary diagnosis	No. patients	Abnormality 5	Abnormality 7	Abnormalities 5 and 7	Balanced rearrangement	Other abnormalities	Normal
No malignancy	18	—	157	—	—	—	—
Hematologic malignancy	167	73	65	63	30	63	41
Hodgkin disease	75	58	65	68	—	66	—
Non-Hodgkin lymphoma	69	106	76	59	—	60	—
Myeloma	22	72	50	—	—	—	—
Other	1	—	—	—	—	—	—
Solid tumor	109	48	56	77	28	71	88
Breast	30	69	66	—	25	—	—
Ovary	12	—	—	—	—	—	—
Prostate	12	—	—	—	—	—	—
Lung	9	—	—	—	—	—	—
Cervix	7	—	—	—	—	—	—
Other	39	51	52	105	31	—	78
Median latency, mo	NA	64	66	65	28*	68	69
IQR, mo	NA	39-114	44-115	45-107	18-49*	38-97	34-115
Range, mo	NA	13-396	3-626	10-276	9-216	12-548	12-440
Total patients, n	294	60	84	60	31	36	23

NA indicates not applicable; —, subgroups with fewer than 5 subjects (medians were not computed for these patients).

*Patients with balanced rearrangements had significantly shorter latency periods than all other cytogenetic groups ($P = .0003$).

Table 9. Association between specific cytogenetic subgroups and prior exposure to various chemotherapy drugs or radiation in patients with t-MDS/t-AML

Therapeutic agent	No. patients (%)*	Abnormality 5 (%)†	Abnormality 7 (%)†	Abnormalities 5 and 7 (%)†	Balanced rearrangement (%)†‡	Other abnormalities (%)†	Normal (%)†	*P*
Alkylator								.19
Yes	240 (82)	44 (18)	73 (30)	52 (22)	24 (10)	30 (13)	17 (7)	
No	53 (18)	15 (28)	10 (19)	9 (17)	7 (13)	5 (9)	7 (13)	
Topoisomerase 2 inhibitor								.013
Yes	115 (39)	16 (14)	29 (25)	27 (23)	18 (16)	19 (17)	6 (5)	
No	177 (61)	43 (24)	54 (31)	34 (19)	13 (7)	16 (9)	17 (10)	
Antimetabolite								.34
Yes	93 (32)	15 (16)	31 (33)	18 (19)	8 (9)	10 (11)	11 (12)	
No	198 (68)	44 (22)	52 (26)	43 (22)	22 (11)	25 (13)	12 (6)	
Antitubulin								.19
Yes	158 (54)	30 (19)	46 (29)	36 (23)	13 (8)	24 (15)	9 (6)	
No	134 (46)	30 (22)	37 (28)	25 (19)	17 (13)	11 (8)	14 (11)	
RT								.79
Yes	182 (60)	40 (22)	46 (25)	40 (22)	20 (11)	23 (13)	13 (7)	
No	121 (40)	21 (17)	38 (31)	25 (21)	11 (9)	15 (12)	11 (9)	

*Numbers in parentheses in this column are percentages within each chemotherapy agent category.
†Numbers in parentheses for all other columns are percentages reported for each row of data according to cytogenetic subgroup.
‡Two patients, one each with abnormal chromosome 5 or 7, are counted only in the balanced rearrangement category because of t(3;21) or inv(16).

patients with AML de novo who had deletions of chromosomes 5 or 7 in the recent Cancer and Leukemia Group B (CALGB) series were 0.3 and 0.5 years despite intensive chemotherapy.[31] In the International Workshop on Balanced Translocations, patients with t-AML and inv(16) had a median survival of only 29 months compared with a median survival of 8 years for patients with AML de novo and inv(16) in the recent CALGB series.[24,31] However, a recent comparison by the Gruppo Italiano Malattie Ematologische Maligne dell'Adulto (GIMEMA) group between patients with APL de novo and t-APL using uniform treatment approaches showed similar outcomes.[32]

Many investigators have associated RT with the subsequent development of t-MDS/t-AML, though its specific contributory role has been debated. Pedersen-Bjergaard et al[33] did not find RT to be a risk factor for the development of t-MDS/t-AML in more than 1500 patients with HD, NHL, or ovarian cancer. A review of balanced rearrangements in the Mitelman database similarly did not support RT exposure as a risk factor for t-MDS/t-AML.[34] Cytogenetic analysis of patients with t-MDS/t-AML from the MD Anderson Cancer Center showed that abnormalities of chromosomes 5 and 7 were found in only 29% of patients exposed to RT alone compared with 72% to 83% of patients after melphalan-, nitrogen mustard–, or nitrosourea-based therapy.[35] In contrast, pretransplantation radiation was found to be a risk factor for t-MDS/t-AML after high-dose therapy and autologous stem cell rescue.[36,37] Although the possible synergistic versus additive roles of RT are still debated, t-MDS/t-AML with characteristic features clearly occurs after exposure to RT alone. Consistently, in each of the previously mentioned series, 10% to 20% of patients had been exposed to RT alone. Our data show no significant clinical or cytogenetic differences between patients acquiring t-MDS/t-AML after RT alone compared with CT alone or CMT. The latency interval, spectrum of cytogenetic aberrations, and prognosis did not differ significantly between our patients receiving RT, CT, or CMT. Other reports suggest an association among RT, t(15;17), and inv(16).[24] In our series, 6 patients with balanced rearrangements had previously been treated with RT alone. Of these patients, 3 had t-AML with t(15;17) and 1 had inv(16). Because t-MDS/t-AML clearly occurs after RT alone and because RT does not accelerate the pace of development of t-MDS/t-AML either alone or as a component of CMT, it is reasonable to postulate that RT acts as

Table 10. Latency intervals after first cytotoxic therapy to first bone marrow dysfunction according to exposure to specific classes of chemotherapy or radiation therapy

Therapeutic agent	No. patients	Median latency, mo	IQR, mo	Range, mo	*P*
Alkylator					
Yes	236	61	36-106	3-524	.72
No	52	62	26-114	10-626	
Topoisomerase 2 inhibitor					
Yes	112	63	34-98	9-396	.53
No	175	60	37-115	3-626	
Antimetabolite					
Yes	92	66	35-106	3-265	.81
No	194	60	35-112	9-626	
Antitubulin					
Yes	155	66	40-107	9-396	.14
No	132	54	28-108	3-626	
RT					
Yes	177	67	39-107	10-626	.08
No	116	53	33-106	3-238	

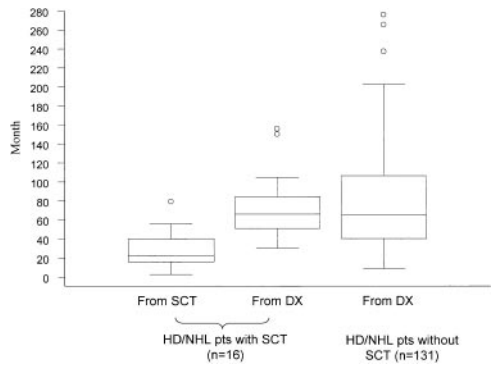

Figure 1. Median latency intervals (horizontal line) with interquartile range (box) and overall range for 16 patients with Hodgkin's disease (HD) or non-Hodgkin lymphoma (NHL) who underwent autologous stem cell transplantation (SCT) compared with 131 patients with HD or NHL who did not receive SCT. For the 16 patients with HD or NHL undergoing SCT, the median latency interval to development of t-MDS/t-AML was 66 months from initial diagnosis and 22 months from SCT. This compares to the 65-month median latency interval from initial diagnosis to t-MDS/t-AML for HD and NHL patients not undergoing SCT. Circles indicate outliers.

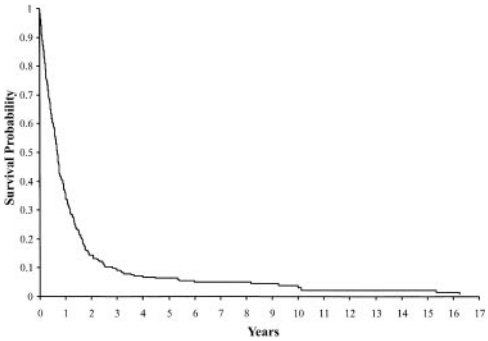

Figure 2. Overall survival from diagnosis of t-MDS/t-AML in 306 patients. The numbers of patients remaining at risk at 1, 2, 3, 4, 5, 7, and 9 years were 102, 41, 24, 17, 15, 9, and 6 patients, respectively.

many other DNA-damaging agents in contributing to therapy-related bone marrow dysfunction. RT is known to damage DNA through the induction of double-strand breaks similar to alkylating agents.[38] This suggests that RT-mediated mutagenicity is no different than the contribution of many chemotherapeutic agents used as part of combination chemotherapy, and it may explain the similar clinical and cytogenetic features after either modality.

We and others[15-17,21,23] had previously identified the association of t-MDS/t-AML involving the *MLL* gene at chromosome band 11q23 with exposure to high cumulative doses of the topoisomerase 2 inhibitor, etoposide, and found that the natural history and clinical features of t-AML characterized by balanced translocations of 11q23 and 21q22 were clearly different from those seen in patients with abnormal chromosome 5 or 7. This was originally identified by Pedersen-Bjergaard and subsequently confirmed by many others.[14,34,39] It also appears that other balanced rearrangements in patients with t-MDS/t-AML constitute a similar syndrome characterized by shorter latency compared with that following alkylator therapy. In our current series, patients with balanced rearrangements had shorter latency periods than patients with all other chromosomal abnormalities (median, 28 vs 64-69 months). Such a short latency has been characteristic of topoisomerase 2 inhibitor-associated t-MDS/t-AML.[16-18,20,40] Of the 10 patients in the current series with a t(11q23), 9 had received a topoisomerase 2 inhibitor (5 doxorubicin, 4 etoposide) in combination with other chemotherapy agents. Among the 8 patients with t(3;21), t(8;21), or other t(21q22), 4 had previously received a topoisomerase 2 inhibitor (2 doxorubicin, 2 doxorubicin and etoposide) in combination with other chemotherapy, and 4 had not received such agents. Five of 6 patients with t(15;17) never received topoisomerase 2 inhibitors, but all 6 received RT, either with (n = 3) or without (n = 3) chemotherapy. Three of the 6 patients with inv(16) received topoisomerase 2 inhibitors combined with other chemotherapy, and 5 of 6 also received RT.

The recent WHO classification emphasizes 2 different therapy-related bone marrow syndromes—one occurring after exposure to alkylating agents or radiation therapy (typically with abnormalities of chromosomes 5 or 7) and the other occurring after exposure to topoisomerase 2 inhibitors (typically with balanced translocations involving 11q23 or 21q22).[13] Unfortunately, the overlapping use of multiple agents and the common use of alkylating agents in 82% of patients precludes more specific analysis of the effects of topoisomerase 2 inhibitors using our current data set. Only 3 patients received topoisomerase 2 inhibitors in the absence of exposure to alkylating agents, and it is not useful to compare the 2 groups in terms of latency or survival.

Our experience with therapy-related leukemia after autologous SCT suggests that the initial cytotoxic therapy for the primary malignancy is more likely to be responsible for t-MDS/t-AML than the actual transplantation procedure itself.[41] This is inferred from the latency interval, which is similar to that observed in other patients treated only with conventional cytotoxic therapy and not with autologous SCT. Others have also reported short latency intervals to bone marrow dysfunction after SCT, though none have compared latency intervals with patients not undergoing SCT.[37,42-44] Traweek et al[42] evaluated 10 patients with morphologically and cytogenetically normal bone marrow at the time of SCT who subsequently acquired clonal karyotypic aberrations. They found that the first cytogenetic changes typical of t-MDS/t-AML occurred at a mean of 1.4 years after SCT, even if morphologic changes had not yet occurred. Retrospective evaluation by another group using sensitive fluorescence in situ hybridization (FISH) assays showed that clonally abnormal cells were detected in pre-SCT cryopreserved bone marrow specimens in all SCT patients who subsequently showed morphologic evidence of t-MDS/t-AML. In contrast, in a pre-SCT sample, FISH revealed clonally abnormal cells in only 3 of 24 patients who did not have t-MDS/t-AML after SCT.[45] Thus, though it is likely that the cytotoxic therapy delivered during SCT is additive to previous genomic damage and may contribute to the etiology by cooperating mutations, there is persuasive evidence that high-dose CT or RT during SCT itself is not the main causative factor of t-MDS/t-AML.

The etiology and specific predisposing features of t-MDS/t-AML remain elusive because, fortunately, only a small fraction of patients exposed to cytotoxic therapy acquire the syndrome. Twenty-eight patients in our series had more than one prior malignancy before diagnosis with t-MDS/t-AML (data not shown). This suggests the presence of a constitutional defect that might predispose to malignancy. We have previously reported that the frequency of an inactivating polymorphism in the *NQO1* gene (NAD(P)H:quinone oxidoreductase) is increased among patients with t-MDS/t-AML.[46] This enzyme is important in detoxifying natural products and has been implicated in benzene-induced hematotoxicity. Other polymorphisms involving detoxifying enzymes have also been reported in patients with t-MDS/t-AML.[47,48] The identification of genes predisposing to t-MDS/t-AML and genes that are most commonly altered by cytotoxic therapies may eventually allow the identification of patients at highest risk.

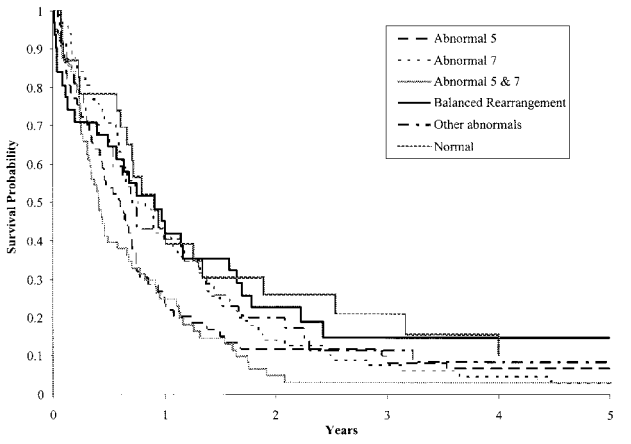

Figure 3. Survival from diagnosis of t-MDS/t-AML by recurring cytogenetic abnormalities. The number of patients remaining at risk at 1, 2 and 4 years, respectively, were as follows: 15, 7, and 4 for abnormal 5; 34, 11, and 3 for abnormal 7; 15, 3, and 1 for abnormal 5 and 7; 14, 7, and 4 for recurring balanced rearrangements; 14, 7, and 3 for other clonal abnormalities; and 10, 6, and 2 for normal karyotype.

Overall the prognosis for patients with t-MDS/t-AML remains poor (Figures 2 and 3). Prevention or avoidance of this late complication of otherwise curative cancer therapies is critical, and primary treatment programs must be continuously re-evaluated to diminish the risk. Further study of the consistent cytogenetic changes induced by specific cytotoxic therapies will lead to the discovery of important genes and gene products that are involved in de novo and therapy-related leukemogenesis and potentially to better therapies.

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References

1. 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. 1977;50:759-770.
2. Rowley JD, Golomb HM, Vardiman JW. Nonrandom chromosome abnormalities in acute leukemia and dysmyelopoietic syndromes in patients with previously treated malignant disease. Blood. 1981;58:759-767.
3. Vardiman JW, Golomb HM, Rowley JD, Variakojis D. Acute nonlymphocytic leukemia in malignant lymphoma: a morphologic study. Cancer. 1978; 42:229-242.
4. Pedersen-Bjergaard J, 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 HLA typing. Blood. 1981;57:712-723.
5. Sandberg AA, Wake N, Kohno S. Chromosomes and causation of human cancer and leukemia, XLVII: severe hypodiploidy and chromosome conglomerations in ALL. Cancer Genet Cytogenet. 1982;5:293-307.
6. Albain KS, Le Beau MM, Vardiman JW, Golomb HM, Rowley JD. Development of dysmyelopoietic syndrome in a hairy cell leukemia patient treated with chlorambucil: cytogenetic and morphologic evaluation. Cancer Genet Cytogenet. 1983;8: 107-115.
7. Pedersen-Bjergaard J, Philip P, Pedersen NT, et al. Acute nonlymphocytic leukemia, preleukemia, and acute myeloproliferative syndrome secondary to treatment of other malignant diseases, II: bone marrow cytology, cytogenetics, results of HLA typing, response to antileukemic chemotherapy, and survival in a total series of 55 patients. Cancer. 1984;54:452-462.
8. Shepard KV, Larson R, Le Beau MM, Leichman L, Levin B. Acute leukemia occurring after radiotherapy and chemotherapy with a nitrosourea, PCNU. Invest New Drugs. 1988;6:121-124.
9. Arthur DC, Bloomfield CD. Banded chromosome analysis in patients with treatment-associated acute nonlymphocytic leukemia. Cancer Genet Cytogenet. 1984;12:189-199.
10. Le Beau MM, Albain KS, Larson RA, et al. 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. J Clin Oncol. 1986;4:325-345.
11. Rubin CM, Arthur DC, Woods WG, et al. Therapy-related myelodysplastic syndrome and acute myeloid leukemia in children: correlation between chromosomal abnormalities and prior therapy. Blood. 1991;78:2982-2988.
12. Thirman MJ, Larson RA. Therapy-related myeloid leukemia. Hematol Oncol Clin North Am. 1996; 10:293-320.
13. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization Classification of Tumours: Tumours of Haematopoietic and Lymphoid Tissues. In: Pathology and Genetics. Lyons, France: IARC Press; 2001:75-106.
14. Pui CH, Relling MV, Rivera GK, et al. Epipodophyllotoxin-related acute myeloid leukemia: a study of 35 cases. Leukemia. 1995;9:1990-1996.
15. Pedersen-Bjergaard J, Philip P. Balanced translocations involving chromosome bands 11q23 and 21q22 are highly characteristic of myelodysplasia and leukemia following therapy with cytostatic agents targeting at DNA-topoisomerase II. Blood. 1991;78:1147-1148.
16. Ratain MJ, Kaminer LS, Bitran JD, et al. Acute nonlymphocytic leukemia following etoposide and cisplatin combination chemotherapy for advanced non-small-cell carcinoma of the lung. Blood. 1987;70:1412-1417.
17. Larson RA, Le Beau MM, Ratain MJ, Rowley JD. Balanced translocations involving chromosome bands 11q23 and 21q22 in therapy-related leukemia. Blood. 1992;79:1892-1893.
18. Super HJ, McCabe NR, Thirman MJ, et al. Rearrangements of the *MLL* gene in therapy-related acute myeloid leukemia in patients previously treated with agents targeting DNA-topoisomerase II. Blood. 1993;82:3705-3711.
19. Thirman MJ, Gill HJ, Burnett RC, et al. Rearrangement of the *MLL* gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. N Engl J Med. 1993; 329:909-914.
20. Roulston D, Espinosa R III, Nucifora G, Larson RA, Le Beau MM, Rowley JD. *CBFA2(AML1)* translocations with novel partner chromosomes in myeloid leukemias: association with prior therapy. Blood. 1998;92:2879-2885.
21. Slovak ML, Bedell V, Popplewell L, Arber DA, Schoch C, Slater R. 21q22 balanced chromosome aberrations in therapy-related hematopoietic disorders: report from an international workshop. Genes Chromosomes Cancer. 2002;33: 379-394.
22. Zhang Y, Strissel P, Strick R, et al. Genomic DNA breakpoints in *AML1/RUNX1* and *ETO* cluster with topoisomerase II DNA cleavage and DNase I hypersensitive sites in t(8;21) leukemia. Proc Natl Acad Sci U S A. 2002;99:3070-3075.
23. Bloomfield CD, Archer KJ, Mrozek K, et al. 11q23 balanced chromosome aberrations in treatment-related myelodysplastic syndromes and acute leukemia: report from an international workshop. Genes Chromosomes Cancer. 2002;33:362-378.
24. Andersen MK, Larson RA, Mauritzson N, Schnittger S, Jhanwar SC, Pedersen-Bjergaard J. Balanced chromosome abnormalities inv(16) and t(15;17) in therapy-related myelodysplastic syndromes and acute leukemia: report from an international workshop. Genes Chromosomes Cancer. 2002;33:395-400.
25. Rubin CM, Larson RA, Anastasi J, et al. t(3;21)(q26;q22): a recurring chromosomal abnormality in therapy-related myelodysplastic syndrome and acute myeloid leukemia. Blood. 1990; 76:2594-2598.
26. Appelbaum FR, Le Beau MM, Willman C. White cells: secondary leukemia. Hematology (Am Soc Hematol Educ Program). 1996:33-47.
27. ISCN. An International System for Human Cytogenetic Nomenclature. In: Mittelman F, ed. New York, NY: Karger Basel; 1995.
28. Kruskal WH, Wallis WA. Use of ranks in one-criterion analysis of variance. J Am Stat Assoc. 1952; 47:583-621.
29. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53:457-481.
30. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient, II: analysis and examples. Br J Cancer. 1977;35:1-39.
31. Byrd JC, Mrozek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with *de novo* acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 2002;100:4325-4336.
32. Pulsoni A, Pagano L, Lo Coco F, et al. Clinicobiological features and outcome of acute promyelocytic leukemia occurring as a second tumor: the GIMEMA experience. Blood. 2002;100:1972-1976.
33. Pedersen-Bjergaard J, Philip P, Larsen SO, et al. 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. Leukemia. 1993;7:1975-1986.
34. Andersen MK, Johansson B, Larsen SO, Pedersen-Bjergaard J. Chromosomal abnormalities in secondary MDS and AML: relationship to drugs and radiation with specific emphasis on the balanced rearrangements. Haematologica. 1998;83: 483-488.
35. Kantarjian HM, Keating MJ, Walters RS, et al. Therapy-related leukemia and myelodysplastic syndrome: clinical, cytogenetic, and prognostic features. J Clin Oncol. 1986;4:1748-1757.
36. Krishnan A, Bhatia S, Slovak ML, et al. Predictors of therapy-related leukemia and myelodysplasia following autologous transplantation for lymphoma: an assessment of risk factors. Blood. 2000;95:1588-1593.
37. Darrington DL, Vose JM, Anderson JR, et al. Incidence and characterization of secondary myelodysplastic syndrome and acute myelogenous leukemia following high-dose chemoradiotherapy and autologous stem-cell transplantation for lymphoid malignancies. J Clin Oncol. 1994;12:2527-2534.
38. Lichter AS, Lawrence TS. Recent advances in radiation oncology. N Engl J Med. 1995;332:371-379.
39. Zhang Y, Poetsch M, Weber-Matthiesen K, et al. Secondary acute leukaemias with 11q23 rearrangement: clinical, cytogenetic, FISH and FICTION studies. Br J Haematol. 1996;92:673-680.
40. Ratain MJ, Rowley JD. Therapy-related acute myeloid leukemia secondary to inhibitors of topoisomerase II: from the bedside to the target genes. Ann Oncol. 1992;3:107-111.
41. Sobecks RM, Le Beau MM, Anastasi J, Williams SF.

Myelodysplasia and acute leukemia following high-dose chemotherapy and autologous bone marrow or peripheral blood stem cell transplantation. Bone Marrow Transplant. 1999;23:1161-1165.

42. Traweek ST, Slovak ML, Nademanee AP, Brynes RK, Niland JC, Forman SJ. Clonal karyotypic hematopoietic cell abnormalities occurring after autologous bone marrow transplantation for Hodgkin's disease and non-Hodgkin's lymphoma. Blood. 1994;84:957-963.
43. Pedersen-Bjergaard J, Pedersen M, Myhre J, Geisler C. High risk of therapy-related leukemia after BEAM chemotherapy and autologous stem cell transplantation for previously treated lymphomas is mainly related to primary chemotherapy and not to the BEAM-transplantation procedure. Leukemia. 1997;11:1654-1660.
44. Lambertenghi Deliliers G, Annaloro C, Pozzoli E, et al. Cytogenetic and myelodysplastic alterations after autologous hemopoietic stem cell transplantation. Leuk Res. 1999;23:291-297.
45. Lillington DM, Micallef IN, Carpenter E, et al. Detection of chromosome abnormalities pre–high-dose treatment in patients developing therapy-related myelodysplasia and secondary acute myelogenous leukemia after treatment for non-Hodgkin's lymphoma. J Clin Oncol. 2001;19:2472-2481.
46. Larson RA, Wang Y, Banerjee M, et al. Prevalence of the inactivating 609C→T polymorphism in the NAD(P)H:quinone oxidoreductase (*NQO1*) gene in patients with primary and therapy-related myeloid leukemia. Blood. 1999; 94:803-807.
47. Allan JM, Wild CP, Rollinson S, et al. Polymorphism in glutathione S-transferase P1 is associated with susceptibility to chemotherapy-induced leukemia. Proc Natl Acad Sci U S A. 2001;98: 11592-11597.
48. Naoe T, Takeyama K, Yokozawa T, et al. Analysis of genetic polymorphism in NQO1, GST-M1, GST-T1, and CYP3A4 in 469 Japanese patients with therapy-related leukemia/myelodysplastic syndrome and de novo acute myeloid leukemia. Clin Cancer Res. 2000;6:4091-4095.
40. Smith SM, Le Beau MM, Huo D, et al. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series [abstract]. Blood. 2001;98:458a.