Chromosome Aberrations and Prognostic Factors in Therapy-Related Myelodysplasia and Acute Nonlymphocytic Leukemia

By Jens Pedersen-Bjergaard, Preben Philip, Severin Olesen Larsen, Grethe Jensen, and Karin Byrsting

Cytogenetic studies of 91 consecutive patients with therapy-related myelodysplasia or overt acute nonlymphocytic leukemia disclosed characteristic defects of chromosome 7 in 48 cases and of chromosome 5 in 21 cases. The chromosome 5 abnormalities were consistently present in all abnormal mitoses at the time of diagnosis, as were the chromosome 7 abnormalities in 45 of the 48 patients. Various abnormalities, primarily of the short arm of chromosome 17, were observed in 13 cases, abnormalities of the long arm of chromosome 21 were observed in 12 cases, and rearrangements of 11q23 were seen in nine cases. Thirteen patients presented a normal karyotype. Previous therapy with alkylating agents, the presence of an initial myelodysplastic phase, and abnormalities of chromosome 7 or 5 were interdependent. Patients with 11q23 rearrangement typically developed overt leukemia of FAB types M4 or M5a without myelodysplasia and with a short latent period. Evaluated by Cox regression analysis, complete remission of the primary malignancy and a malignant lymphoma as primary tumor were the two most important and independent prognostic factors indicating a longer survival \( (P= .008) \). In addition, the platelet count at diagnosis was a significant prognostic factor \( (P= .01) \). For the subgroup of 62 patients with myelodysplasia, the number of chromosome aberrations, the percentage of blasts in the bone marrow, and the hemoglobin level were other significant and independent prognostic factors \( (P= .05, .05, \text{and } .004, \text{respectively}) \). The most important predictive factor for a favorable response to intensive antileukemic chemotherapy in overt leukemia was the absence of a preceding myelodysplastic phase \( (P= .0014) \).

For many years therapy-related myelodysplasia (t-MDS) and therapy-related acute nonlymphocytic leukemia (t-ANLL) have been recognized as the most serious long-term complications of current cancer therapy. In previous studies of patients with Hodgkin’s disease, advanced age has been identified as the predominant predisposing factor.\(^1,5\) As in many other types of tumor, treatment with alkylating agents has been disclosed as the most important direct causal factor.\(^4,6\)

Other concurrent studies have discussed the characteristic chromosome abnormalities observed in 80% to 90% of patients with t-MDS or t-ANLL.\(^7,22\) The predominant aberrations are loss of whole chromosomes 7 and 5 or various parts of the long arms of these two chromosomes. In addition, nonrandom abnormalities have been reported, particularly of chromosomes 17 and 21.

It is possible that the generally short survival of patients is the reason for the as yet scarce discussion of prognostic factors in t-MDS and t-ANLL. However, the combined fact that some patients with t-MDS may survive for prolonged periods of time,\(^15,16\) that others with overt t-ANLL may respond to intensive chemotherapy as do patients with de novo leukemia\(^15,24,25\) and may become long-term survivors, and that bone marrow transplantation is now in widespread clinical use, makes re-evaluating prognostic factors in t-MDS and t-ANLL an urgent issue.

Whereas the presence of characteristic chromosome aberrations has been shown to be of major importance in the diagnosis of t-MDS,\(^7,8,11,15\) their role as a prognostic factor has so far remained controversial. We have previously presented data suggesting that the number of chromosome aberrations in t-MDS and t-ANLL is a prognostic factor.\(^15,19\) As in some studies of the de novo myelodysplastic syndromes,\(^26-29\) however, another major study has not confirmed our findings,\(^27\) and in de novo myelodysplasia and acute nonlymphocytic leukemia, it is the specific chromosome aberrations rather than their number that have been proposed as an important prognostic factor.\(^30-33\)

This study extends our experience to 91 consecutive cases of t-MDS and t-ANLL examined cytogenetically. It was undertaken to re-evaluate the frequency of specific chromosome abnormalities, to evaluate whether they are consistently present in all abnormal mitoses at diagnosis or only in a subclone in the individual patient, to study cytogenetic evolution during the course of the disease, and to search for a possible relationship between specific chromosome aberrations and important clinical and pathologic parameters. Finally, a Cox regression analysis was carried out to examine the importance of cytogenetic parameters as compared with major clinical and pathologic findings as independent prognostic factors in patients with t-MDS and t-ANLL.

PATIENTS AND METHODS

Patient characteristics and cytologic classification. During the period between 1987 and 1989, 21 new cases of t-MDS or t-ANLL were diagnosed at our institution in patients previously treated with radiotherapy and/or chemotherapy (Table 1). Seven patients had previously received treatment for malignant lymphomas; six, for breast cancer; seven, for various other malignancies; and one patient had received treatment with azathioprine for sarcoidosis. The 21 new cases of t-MDS or t-ANLL, together with the 70 cases for which clinical and complete cytogenetic data have been reported previously,\(^5,15,19,36\) comprise our total experience for the period between 1976 and 1989. Overt t-ANLL was classified according to...
Table 1: Clinical Characteristics, Bone Marrow Cytology, and Cytogenetic Findings at Diagnosis in a New Series of Patients With t-MDS or t-ANLL

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/ Sex</th>
<th>Primary Tumor Histology/ Stage</th>
<th>Type of Treatment for Primary Tumor (mos. duration)</th>
<th>Status of Primary Tumor</th>
<th>Survival From MDS or ANLL (mos)</th>
<th>Karyotype of BM C</th>
<th>Andeils. Chemother. response</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>76/M</td>
<td>Hodgkin NS/HA</td>
<td>Me + VCR + Pred + Pro (16)</td>
<td>CR</td>
<td>34 3 3</td>
<td>46,XY, -5, -6, -15, -16, -17, +del(6)(p23; q23), +del(7)(q23), +del(11)(q15; q18) (12q21; q17; 17q12; 17q21) = 13/45, as stemline with -18, del(16) (12q) and without -18, +del(16) = 7-45, as stemline with (8)(q) = 5</td>
<td>28/1</td>
</tr>
<tr>
<td>90</td>
<td>43/F</td>
<td>Hodgkin NS/HV</td>
<td>CCNU + Vb + Pro + Pred (8)</td>
<td>PR</td>
<td>88 3 3</td>
<td>46,XX,del(13)(q12;q21) (13q12-13q21) = 13/45, as stemline with -18, del(16) (12q) and without -18, +del(16) = 7-45, as stemline with (8)(q) = 5</td>
<td>10/30</td>
</tr>
<tr>
<td>91</td>
<td>67/F</td>
<td>Non-Hodgkin NM/HIA</td>
<td>Ctx (38)</td>
<td>CR</td>
<td>38 36+ 36+</td>
<td>46,XX, -7</td>
<td>18/2</td>
</tr>
<tr>
<td>92</td>
<td>60/F</td>
<td>Non-Hodgkin DW/HIA</td>
<td>VCR + Pred + Sm (2)</td>
<td>PR</td>
<td>82 6 6</td>
<td>46,XX,del(13)(q11q14) (13q11-13q14) = 13/45, as stemline with -18, del(16) (12q) and without -18, +del(16) = 7-45, as stemline with (8)(q) = 5</td>
<td>21/40</td>
</tr>
<tr>
<td>93</td>
<td>49/M</td>
<td>Non-Hodgkin NM/HIA</td>
<td>VCR + Pred + Sm (2)</td>
<td>PR</td>
<td>159 2 9</td>
<td>M4 46,XY, -7, +8, (1q7p) (1q7p13-1q7p36) = 13/45, as stemline with -18, del(16) (12q) and without -18, +del(16) = 7-45, as stemline with (8)(q) = 5</td>
<td>29/0</td>
</tr>
<tr>
<td>94</td>
<td>72/M</td>
<td>Non-Hodgkin lymphoblast/IE</td>
<td>X-rays rhinopharynx and neck, 36 Gy</td>
<td>CR</td>
<td>55 18+ 18+</td>
<td>46,XX, -7</td>
<td>24/0</td>
</tr>
<tr>
<td>95</td>
<td>65/M</td>
<td>Non-Hodgkin lymphoblast/IE</td>
<td>CHOP (5)</td>
<td>CR</td>
<td>32 13+</td>
<td>44,XX,del(6)(q13q33), (13q13-13q33) = 13/45, as stemline with -18, del(16) (12q) and without -18, +del(16) = 7-45, as stemline with (8)(q) = 5</td>
<td>29/0</td>
</tr>
<tr>
<td>96</td>
<td>74/F</td>
<td>Multiple myeloma lgG</td>
<td>MaPhalan + Pred (12)</td>
<td>PR</td>
<td>17 1 1</td>
<td>44,XX,del(6)(q13q33), (13q13-13q33) = 13/45, as stemline with -18, del(16) (12q) and without -18, +del(16) = 7-45, as stemline with (8)(q) = 5</td>
<td>14/1</td>
</tr>
<tr>
<td>97</td>
<td>63/F</td>
<td>Breast cancer duct cell/ disseminated</td>
<td>X-rays Mc Wharter, 40.7 Gy</td>
<td>Ctx (15)</td>
<td>137 3 1</td>
<td>45,XX, -7</td>
<td>12/6</td>
</tr>
<tr>
<td>98</td>
<td>61/F</td>
<td>Breast cancer duct cell/ disseminated</td>
<td>X-rays Mc Wharter, 40.7 Gy</td>
<td>Ctx (15)</td>
<td>137 3 1</td>
<td>45,XX, -7</td>
<td>12/6</td>
</tr>
<tr>
<td>99</td>
<td>68/F</td>
<td>Breast cancer duct cell/ disseminated</td>
<td>X-rays Mc Wharter, 32. Gy</td>
<td>Ctx (15)</td>
<td>137 3 1</td>
<td>45,XX, -7</td>
<td>12/6</td>
</tr>
<tr>
<td>100</td>
<td>67/F</td>
<td>Breast cancer duct cell/ disseminated</td>
<td>X-rays Mc Wharter, 32. Gy</td>
<td>Ctx (15)</td>
<td>137 3 1</td>
<td>45,XX, -7</td>
<td>12/6</td>
</tr>
<tr>
<td>101</td>
<td>42/M</td>
<td>Breast cancer duct cell/ disseminated</td>
<td>X-rays Mc Wharter, 30 Gy</td>
<td>Ctx (15)</td>
<td>137 3 1</td>
<td>45,XX, -7</td>
<td>12/6</td>
</tr>
<tr>
<td>102</td>
<td>39/F</td>
<td>Breast cancer duct cell/ disseminated</td>
<td>X-rays Mc Wharter, 12.5 Gy</td>
<td>Ctx (15)</td>
<td>137 3 1</td>
<td>45,XX, -7</td>
<td>12/6</td>
</tr>
<tr>
<td>103</td>
<td>54/F</td>
<td>Small-cell lung cancer/ extensive</td>
<td>X-rays Mc Wharter, 20 Gy</td>
<td>Ctx (15)</td>
<td>137 3 1</td>
<td>45,XX, -7</td>
<td>12/6</td>
</tr>
</tbody>
</table>

(Continued on following page)
the FAB recommendations,\textsuperscript{17} and t-MDS was classified as previously described.\textsuperscript{8,19} No distinction is now being made between preleukemia and the acute myeloproliferative syndrome.

\textbf{Cytogenetic studies.} Cytogenetic investigations were carried out using GTL banding technique after a 1- to 2-day culture of a bone marrow sample. Results were expressed in accordance with the International System for Human Cytogenetic Nomenclature (ISCN).\textsuperscript{38} Furthermore, patients were classified as NN, AN, or AA based on the presence of normal mitoses only, a mixture of normal and abnormal mitoses, or abnormal mitoses only, as previously reported for de novo ANLL.\textsuperscript{39} The number of chromosome aberrations was estimated, assessing a translocation between two chromosomes in all abnormal mitoses, or abnormal mitoses only, as previously reported for de novo ANLL.\textsuperscript{39} The statistical significance of differences in frequencies between groups of patients was assessed with Fisher's exact test (two-sided), and differences in the latent period for development of t-MDS or t-ANLL between groups of patients were evaluated by Wilcoxon's two-sample test (two-sided).

\textbf{RESULTS}

Clinical data and results of cytogenetic investigation of the bone marrow of 21 new cases of t-MDS and t-ANLL are shown in Table 1. Six patients showed loss of whole chromosome 7, one patient loss of chromosome 5, five patients loss of various parts of the long arm of chromosome 7, and two patients loss of parts of the long arm of chromosome 5. Rearrangement of band 11q23 was observed in five patients. Two of our new patients with t-MDS showed deletions of 13q, including band 13q14, as the only abnormality, and two patients showed a t(8;21)(q22;q22), one of these as part of a complex karyotype also including a del(7)(q32). In some recent cases, daunorubicin was replaced by aclacinomycin in an equivalent dose. In patients obtaining a complete remission, an intensification program composed of alternating courses of daunorubicin, cytosine arabinoside, amascrine, and etoposide was administered for a period of 6 months.

\textbf{Statistical methods.} Survival was calculated from the first diagnosis of t-MDS or t-ANLL and assessed by a Kaplan-Meier estimate.\textsuperscript{40} The prognostic significance of various clinical, pathologic, and cytogenetic data for survival was evaluated by the Cox proportional hazards model\textsuperscript{41} using the BMDP program.\textsuperscript{42} All recorded factors (Table 2) were included in the multivariate analysis initially, and a step-down procedure was adopted by removal of the least significant factor and repetition of the analysis until only those factors that had significant prognostic influence ($P < 0.05$) were retained. For each factor, a regression coefficient was estimated as previously reported.\textsuperscript{4} The statistical significance of differences in frequencies between groups of patients was assessed with Fisher's exact test (two-sided), and differences in the latent period for development of t-MDS or t-ANLL between groups of patients were evaluated by Wilcoxon's two-sample test (two-sided).

\begin{table}[ht]
\centering
\caption{Clinical Characteristics, Bone Marrow Cytology, and Cytogenetic Findings at Diagnosis in a New Series of Patients With t-MDS or t-ANLL (Cont'd)}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline
Case No. & Age & Sex & Primary Tumor & Histology/Stage & Type of Treatment for Primary Tumor (mo. duration) & Status of Primary Tumor & Time to Development of MDS or ANLL (mo.) & Survival From MDS or ANLL (mo.) & FAB Type & Overv Type & Karyotype of BMC & Mitoses (abnormal/normal) & Antileuk. Chemoth. (type or response) \\
\hline
104 & 43/F & Thymoma & Ctx + CCNU + VCR + Pred (18) & CR & 94 & 3 & 4 & M1 & 47,XXX, t(8;21) (q22;q22) & 15/20 & Ad + AraC/ NR \\
105 & 32/M & Testicular cancer & Cis-Pi + Bleo + VP-16 + VCR (8) & CR & 62 & 6 & M4 & 46,XY & 0/14 & — \\
106 & 38/M & Testicular cancer & Cis-Pi + Bleo + VP-16 + VCR (8) & CR & 90 & 2 & M2 & 46,XY, t(8;21) (q22;q22) & 26/26 & Ad + AraC/ NR \\
107 & 69/M & Laryngeal cancer & X-rays neck, 63 Gy & CR & 161 & 1 & M5a & 47,XY, t(11;19) (q23.3; q13.2) & 22/4 & — \\
108 & 69/M & Urinary bladder cancer & X-rays urinary bladder, 40 Gy & PD & 29 & 2 & 2 & 45,XY, t(12;11) (p13; q21) & 20/2 & — \\
109 & 44/F & Sarcomedosteal tumor & Mts (20) & CR & 128 & 4 & M1 & 46,XX & 0/26 & Ad + AraC/ NR \\
\hline
\end{tabular}
\end{table}

Abbreviations: BMC, bone marrow cells; NS, nodular sclerosis; MM, nodular mixed; DWDL, diffuse well-differentiated lymphocytic; LPDL, nodular poorly differentiated lymphocytic; Ma, medullary thymic; VCR, vincristine; Pred, prednisone; Pro, proparbazine; Str, streptonigrin; Ctx, cyclophosphamide; CHOP, cyclophosphamide + doxorubicin + vincristine + prednisone; Cbl, chlorambucil; Adm, adriamycin; 5FU, 5-fluouracil; Cis-Pi, cisplatin; HDM, hexamethylmelamine; Mts, methotrexate; Mit, mitomycin; Tam, tamoxifen; CCNU, lomustine; Bleo, bleomycin; Vbi, vindesine; VP16, etoposide; Ams, amascrine; AraC, cytosine arabinoside; AMSA, semustine; VAMP, vincristine + 6-mercaptopurine + methotrexate + prednisone; Epi, 4'-episodarabulin; HD, high dose; CR, complete remission; PR, partial remission; PD, progressive disease; NR, no response; DMR, daunorubicin.
Table 2. Cox Regression Analysis of Prognostic Factors for 91 Cases of t-MDS and t-ANLL

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>-0.003</td>
<td>0.011</td>
<td>0.81</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>-0.368</td>
<td>0.262</td>
<td>0.16</td>
</tr>
<tr>
<td>Primary malignancy</td>
<td>0.628</td>
<td>0.239</td>
<td>0.008</td>
</tr>
<tr>
<td>(lymphoma/others)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previously treated with alkylating agents (Y/N)</td>
<td>0.413</td>
<td>0.358</td>
<td>0.24</td>
</tr>
<tr>
<td>Myelodysplasia observed</td>
<td>-0.084</td>
<td>0.381</td>
<td>0.83</td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>-0.016</td>
<td>0.009</td>
<td>0.07</td>
</tr>
<tr>
<td>Granulocytes (x 10^9/L)</td>
<td>0.001</td>
<td>0.003</td>
<td>0.78</td>
</tr>
<tr>
<td>Thrombocytes (x 10^9/L)</td>
<td>-0.007</td>
<td>0.003</td>
<td>0.013</td>
</tr>
<tr>
<td>Marrow cellularity increased</td>
<td>0.245</td>
<td>0.167</td>
<td>0.15</td>
</tr>
<tr>
<td>Marrow blasts + promyelocytes (%)</td>
<td>0.002</td>
<td>0.012</td>
<td>0.87</td>
</tr>
<tr>
<td>Cytogenetics (NN/AN/AA)</td>
<td>0.124</td>
<td>0.200</td>
<td>0.54</td>
</tr>
<tr>
<td>Chromosome 5 and/or 7 abnormal (Y/N)</td>
<td>0.363</td>
<td>0.298</td>
<td>0.23</td>
</tr>
<tr>
<td>Cytogenetics (no. of aberrations)</td>
<td>0.057</td>
<td>0.032</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>(0.092)</td>
<td>(0.046)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parenthesis represent estimates based exclusively on patients with MDS (n = 62).

Loss of a whole chromosome 7 was the most frequently observed abnormality present in 32 patients (Figs 1 and 2), in 30 as a consistently present aberration seen in all abnormal mitoses studied. In addition, six patients had lost the whole long arm of a chromosome 7 as a result of an unbalanced translocation, and 10 showed unbalanced translocations or apparently terminal deletions with loss of various parts of the long arm of a chromosome 7. Break point for the deletions was most often 7q22; the critical region always deleted comprising bands 7q32 to 7qter. In 15 of the 16 patients, the defects of 7q were consistently present aberrations.

Six patients had lost a whole chromosome 5 and three patients the whole long arm of a chromosome 5 as a result of an unbalanced translocation (Fig 1). Twelve patients showed interstitial deletion of various parts of the long arm of a chromosome 5. The proximal break point varied between bands 5q13 and 5q22 and the distal break point between bands 5q31 and 5q35, indicating a critical region from 5q22 to 5q31. All the abnormalities of chromosome 5 were consistently present.

Nine patients showed rearrangements of band 11q23, including the classic t(9;11)(p21;q23) in four cases (Figs 1 and 2). Five patients with 11q23 rearrangement belong to our new series of cases, whereas data for four patients have been presented previously. Among these, cases 66 and 76 were originally considered as having a normal karyotype. However, a reexamination of new preparations showed a t(9;11)(p21;q23) in all 41 mitoses in case 66 and in all 24 mitoses in case 76. In seven of the patients, the 11q23...
rearrangement was observed as the only abnormality present, and in eight cases, it was a consistently present aberration. Other consistently present aberrations (Fig 1) include monosomy of chromosomes 17 and 18, trisomy q1q, rearrangements of 3q, 17p aberrations, and rearrangements of 21q with break point at band 21q22. Generally, loss of whole chromosomes predominated as consistently present aberrations over gain of whole chromosomes (Fig 1).

Inconsistently present aberrations at diagnosis as shown in Fig 2 differed markedly from consistently present aberrations (Fig 1). As far as numerical aberrations are concerned, gain of whole chromosomes predominated over loss. Four patients presented trisomy 8, and only two patients had lost a whole chromosome 7. Among structural abnormalities, three patients showed rearrangement with loss of the short arm of chromosome 7 and three trisomy for the long arm of chromosome 17. Only one patient showed rearrangement with loss of a part of the long arm of chromosome 7. Monosomy of chromosome 5 or loss of parts of the long arm were never observed as inconsistently present aberrations. Evolutionary aberrations during the course of the disease were observed in 12 patients (Fig 2) of 46 reexamined during the course of the disease. Time from diagnosis to reexamination varied between 3 weeks and 5 years, according to the clinical course of the disease. Like the inconsistently present aberrations, the evolutionary aberrations differed markedly from the consistently present aberrations. Abnormalities of chromosome 5 were not observed as evolutionary events. Only one patient with overt leukemia, who initially presented a normal karyotype and obtained a complete remission after daunorubicin and cytosine arabinoside at relapse, presented a characteristic unbalanced translocation: −7,+t(1q;7p) with loss of the long arm of a chromosome 7.

In our total series of 91 patients, 40 patients had previously been treated for malignant lymphomas, five patients for other hematologic malignancies, 45 patients for a solid tumor, and one patient for a benign disease. At the diagnosis of t-MDS or t-ANLL, the primary disease was in complete remission in 57 patients, whereas 34 had an active primary tumor, and in many cases in an advanced stage with bone marrow involvement. Eleven patients had previously been treated with alkylating agents alone, and 63 patients had been receiving alkylating agents plus other types of therapy, including radiotherapy plus chemotherapy in 33 patients. Ten patients had received radiotherapy alone, three patients radiotherapy plus chemotherapy not including alkylating agents, and four patients chemotherapy alone without alkylating agents.

At diagnosis, 62 patients presented with MDS, whereas 29 patients had overt leukemia of the FAB subtypes shown in Table 3. The bone marrow cellularity was increased in 68 patients, including the 29 patients with overt leukemia. Eight patients with MDS had a normal cellularity and 15 with MDS, a decreased cellularity. During follow-up, 26 patients with t-MDS developed overt leukemia (Table 3). Close correlations were observed between previous treatment with alkylating agents and the presence of a myelodysplastic phase (P = .00007, Table 4), between previous treatment with alkylating agents and chromosome 5 and/or chromo-

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>MDS Present</th>
<th>MDS Absent</th>
<th>Chromosome 5 and/or 7 Abnormal</th>
<th>Chromosome 5 and 7 Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>58*</td>
<td>16*</td>
<td>53†</td>
<td>21†</td>
</tr>
<tr>
<td></td>
<td>4*</td>
<td>13*</td>
<td>4†</td>
<td>12†</td>
</tr>
</tbody>
</table>

Table 3. Bone Marrow Cytology at Diagnosis and During Follow-Up of 91 Patients With t-MDS and t-ANLL

some 7 abnormalities (P = .00007, Table 4), and between the presence of a myelodysplastic phase and chromosome 5 and/or chromosome 7 abnormalities (P = .000001, Table 4).

The latent period for development of leukemia for the nine patients with involvement of 11q23 was often short (median, 24 months), as compared with 49 months for 73 patients without abnormalities of chromosome 11. Evaluated by Wilcoxon’s two-sample test (two-sided), the differences in latent periods between the two groups of patients are significant (P = .04). Seven of nine cases with rearrangement of 11q23 presented with overt leukemia, and seven of eight overt leukemias were of FAB subtypes M4 or M5a.

Results of a Cox regression analysis of prognostic factors for survival from diagnosis of t-MDS or t-ANLL for all 91 patients are shown in Table 2. Clinical findings, basic laboratory results, and results of bone marrow cytology and cytogenetic studies are included in the model. The two most important and independent factors indicating a favorable prognosis were complete remission of the primary malignancy and a malignant lymphoma as primary tumor (P = .008). Apart from these, only the thrombocyte count at diagnosis was an independent prognostic factor (P = .013). When evaluated separately for 62 patients with MDS, the hemoglobin concentration, the percentage of blasts and promyelocytes in the bone marrow, and the number of chromosome aberrations were other independent and significant prognostic factors (P = .004, P = .046, and P = .048 respectively). No other factor approached significance.

Table 4. Relationship Between Previous Therapy With Alkylating Agents, Presence of a Myelodysplastic Phase, and Abnormalities of Chromosomes 5 and/or 7

<table>
<thead>
<tr>
<th>AAs Present</th>
<th>No AAs Present</th>
<th>Chromosome 5 and/or 7 Abnormal</th>
<th>Chromosome 5 and 7 Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS Present</td>
<td>58*</td>
<td>16*</td>
<td>53†</td>
</tr>
<tr>
<td>MDS Absent</td>
<td>4*</td>
<td>13*</td>
<td>4†</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chromosome 5 and/or 7 Abnormal</th>
<th>Chromosome 5 and/or 7 Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>53†</td>
<td>21†</td>
</tr>
</tbody>
</table>

Abbreviations: AA, alkylating agents.

*P = .00007, Fisher’s exact test, two-sided.
†P = .00007, Fisher’s exact test, two-sided.
‡P = .000001, Fisher’s exact test, two-sided.
The importance of the prognostic factors for prediction of survival in patients with t-MDS or t-ANLL is shown in Fig 3. The observed survival is presented as a Kaplan-Meier estimate together with the predicted most favorable and most unfavorable prognosis, taking into consideration the three prognostic factors: remission status of the primary tumor, previous malignant lymphoma or other disease, and thrombocyte count at diagnosis (in our study, varying between $250 \times 10^9/L$ and $5 \times 10^9/L$). Considerable variation is observed, for instance, in the predicted median survival (2.8 to 37 months).

After overt t-ANLL had developed, intensive antileukemic chemotherapy was attempted in 36 patients. Seventeen of these obtained a complete remission (Table 5). The strongest predictive factor for a favorable response was absence of a myelodysplastic phase of the disease ($P = .0014$).

**DISCUSSION**

The present study emphasizes that in most cases of t-MDS or t-ANLL, the bone marrow cells show characteristic clonal cytogenetic abnormalities, predominantly loss of whole chromosomes 5 or 7 or various parts of the long arms of these two chromosomes. Using a new simple classification of clonal chromosome aberrations as consistently or inconsistently present at diagnosis, and comparing the results obtained with the cytogenetic evolution during the course of the disease (Figs 1 and 2), an abnormal chromosome 5 was always consistently present at diagnosis, as were the chromosome 7 abnormalities in most of the patients. Together with the recent mapping of several genes for important hematopoietic growth factors and growth factor receptors to the critical deleted bands on the long arm of chromosome 5, these findings suggest that at least the chromosome 5 defects are directly involved in the pathogenesis of MDS and ANLL. In addition, as previously discussed, cytogenetic abnormalities primarily of chromosomes 5 and 7 are of essential diagnostic importance, particularly in the early stages of t-MDS with a hypoplastic bone marrow and without increased percentage of blasts. Of 62 patients with t-MDS in our study, 15 belong to this category (Table 3).

The marked general difference between all consistently present aberrations and the inconsistent or evolutionary aberrations, as evident by comparing Figs 1 and 2, supports our distinction between the two types of chromosome abnormality and our suggestion that the consistently present aberrations most often represent primary cytogenetic events during leukemogenesis.

Other previously described characteristic aberrations, such as loss of a whole chromosome 17 or 18 and rearrangements of the short arm of chromosome 17 and the long arm of chromosome 21 at band 21q22, were also most often demonstrated to be consistently present aberrations (Fig 1). In addition, the present study confirmed 11q23 rearrangements as a new nonrandom cytogenetic abnormality of t-ANLL, first reported recently. It is striking that in eight of nine patients this abnormality was observed as a consistently present aberration and only as inconsistently present in one patient, who also presented a deletion of the long arm of chromosome 7. Furthermore, the 11q23 rearrangement was the only abnormality present in seven of the nine patients. Equally surprising was the short latent period for development of t-ANLL, often observed in these patients, and the fact that seven of the nine patients presented in overt leukemia without a myelodysplastic phase. As previously discussed, all these facts suggest a different pathogenetic mechanism for development of t-ANLL with 11q23 rearrangements as compared with cases with defects of chromosomes 5 and 7. Two new cases of t-MDS, both with a short survival, presented deletions of the long arm of chromosome 13, including band 13q14. The deletions occurred alone as consistently present aberrations in both cases. Deletions including 13q14 have previously been observed in patients with myeloid disorders, for instance de novo myelodysplasia, and 13q14 carries the recessive gene of putative importance in retinoblastoma.

In patients previously treated with alkylating agents, almost all observed cases of t-MDS and t-ANLL must be considered as directly induced by therapy. For example in Hodgkin's disease, relative risks (ratio of observed:expected cases) of 117 to 133 have been observed. This indicates that statistically, only one case in 117 to 133 cases of t-ANLL related to treatment with alkylating agents can be expected to arise de novo. This high specificity is interesting in relation to our finding of close correlations between previous therapy with alkylating agents, the presence of a myelodysplastic phase, and abnormalities of chromosome 5 or 7 (Table 4). Only a minor group of patients with de novo ANLL share the same characteristics. By comparison, in patients previ-
ously treated with high voltage radiotherapy and chemotherapy without alkylating agents, the characteristics observed were as in de novo ANLL. Thus, only 4 of 17 presented a myelodysplastic phase and abnormal chromosomes 5 or 7 (Table 4). This is consistent with the fact that no substantially increased risk of t-MDS and t-ANLL has been observed after chemotherapy without alkylating agents, or high-voltage radiotherapy. Therefore, the dominant number of leukemias observed in this group of patients could represent randomly occurring cases of de novo ANLL.

Our extended series of patients with t-MDS or t-ANLL demonstrates for the first time that the two most important and independent prognostic factors are the remission status of the primary tumor and whether the primary tumor is a malignant lymphoma or another disease (Table 2). The significance of these two prognostic factors, together with the thrombocyte count, for prediction of survival is shown in Fig 3. The very poor prognosis for patients with an active primary malignancy has an obvious biologic basis and is a serious warning against treating such patients with intensive antileukemic chemotherapy. Furthermore, in any future evaluation of prognostic factors, it will always be necessary to take into consideration the remission status of the primary malignancy. What is more surprising is the more favorable prognosis for patients with t-MDS or t-ANLL after Hodgkin's disease and non-Hodgkin's lymphomas, as compared with other primary tumors. The most likely explanation is an early diagnosis of t-MDS in the lymphoma patients in our institution, the consequence of a close follow-up with routine blood counts combined with cytogenetic examination of the bone marrow in all cases developing refractory cytopenia. So far, this procedure has not been fully adopted in the follow-up of patients treated for other malignancies.

Thrombocytopenia at diagnosis and, for patients with t-MDS, anemia at diagnosis were likewise significant and independent prognostic factors (Table 2). These findings are in agreement with the clinical observation that patients who at diagnosis are found to require repeated transfusions usually have a short survival. For patients with t-MDS, the number of chromosome aberrations and the percentage of blasts in the bone marrow also reached significance as independent prognostic factors. The significance of chromosome aberrations as an independent prognostic factor, which has become evident for patients with de novo ANLL, has until recently been more questionable for patients with de novo MDS, as well as for cases of t-MDS and t-ANLL. In some studies of de novo MDS, the number of chromosome aberrations has been shown to be a predictor of prognosis and leukemic transformation. However, this finding has not been confirmed by other investigators. In a recently published extensive study of 247 patients with MDS, however, the Sixth International Workshop on Chromosomes in Leukemia demonstrated a significant difference in survival for patients classified as NN, AN, or AA, as well as for patients with normal, simple, and complex chromosome aberrations. The basis of survival between patients with a normal karyotype or a single aberration, and patients with multiple aberrations. However, the cytogenetic results were not evaluated in a multiregression analysis together with other prognostic factors, and in another major study comprising 63 cases of t-MDS or t-ANLL, the clinical course and survival were not related to cytogenetic characteristics. The present study of the subgroup of 62 patients with t-MDS clearly demonstrates that the number of chromosome aberrations is an independent prognostic factor (Table 2). However, we did not find any specific aberration as a prognostic predictor, and the NN-AN-AA classification did not predict survival in our study (Table 2).

In this study the percentage of blasts and promyelocytes in the bone marrow at diagnosis of t-MDS was another independent prognostic factor. This observation is consistent with the experience in de novo MDS. Here, the two FAB subtypes refractory anemia with excess of blasts and refractory anemia with excess of blasts in transformation, both characterized by a high percentage of bone marrow blasts in almost all series, experience a particularly short survival. In most series of overt t-ANLL, intensive antileukemic chemotherapy has previously resulted in only a few complete remissions. However, it has been suggested that specific cytogenetic subtypes of t-ANLL have a better prognosis. The results in the present study, in which 17 of 36 patients obtained a complete remission (Table 5), clearly indicate that patients presenting in overt leukemia without myelodysplasia achieve the best response to intensive chemotherapy. Prognostic factors for t-MDS and t-ANLL are of increasing interest because allogeneic bone marrow transplantation now seems to offer a chance of cure in this type of patient.

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