Prognostic Impact of Cytogenetic Abnormalities in Patients With De Novo Acute Nonlymphocytic Leukemia

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Detailed cytogenetic analyses were performed on specimens from 198 patients with de novo acute nonlymphocytic leukemia (ANLL), including high-resolution banding studies in 79 patients. One hundred ninety-two patients received induction therapy with daunorubicin and cytosine arabinoside (Ara-C) with an overall complete response rate (CR) of 63%. Responding patients received repetitive cycles of Ara-C-based intensification therapy. Clonal abnormalities were detected in 65% of the patients with specimens adequate for cytogenetic analysis. Certain cytogenetic changes were closely associated with French-American-British (FAB) morphology, age, and outcome: t(8:21) (closely associated with FAB M2), t(15:17) (associated with FAB M3), and abn 16q22 (associated with FAB M4EOS) tended to occur in younger patients and were associated with favorable outcomes in terms of both CR rate and long-term disease-free survival. In contrast, 19% of patients who had 5q- or 7q- and seven patients with trisomy 8 were older, had a poor prognosis, and usually failed to achieve remission (CR) because of chemotherapy-resistant leukemia. The adverse effect on CR rate and duration in this group of patients was independent of age, and there was no association with particular morphologic subtypes. These data suggest that cytogenetic findings should influence future therapeutic choices.

In particular, patients with abnormalities associated with poor responses may be considered for investigational approaches and may also provide insights into mechanisms of drug resistance.

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263
including ultrastructural peroxidase staining, as well as immunologic assessment of cell surface antigens with monoclonal antibodies directed against myeloid antigens, were used to confirm the myeloid derivation of the blasts in patients with morphologically undifferentiated acute leukemia (FAB M-0).

Chromosome analyses were done on aspirated bone marrow or, occasionally on bone marrow biopsy core and/or unstimulated peripheral blood as previously described.6 Mitoses were harvested after a short (usually 24 hours) culture period. In addition, methotrexate synchronized 24-hour cultures were examined in 79 patients.24 In many of the latter, direct preparations were also studied. Chromosomes were Q banded using quinacrine fluorescence or G-banded using Wright's stain.23 Mitoses were analyzed from duplicate photographs. Whenever possible, at least 20 mitoses were analyzed. Karyotypes were assigned according to the recommendations of the International System for Human Cytogenetic Nomenclature.24 The observation of a minimum of two mitoses with an identical structural rearrangement or extra chromosome or three cells with the same missing chromosome was evidence for the existence of an abnormal clone. When more than one recurring chromosome abnormality was observed in a patient, we characterized the karyotype according to a single change in the hierarchial order shown in Table 1.25 For example, a patient who had an abnormal clone containing both t(15;17) and trisomy 8, was categorized only as a t(15;17) patient. Thus, each patient had a unique classification, although many patients had multiple abnormalities.

Comparisons of remission rates were done by chi-square analysis. The Kaplan-Meier procedure was used for comparisons of survival and CR duration. Duration of CR was calculated from the date of achieving CR until relapse; deaths during CR were considered censored observations. Survival was calculated from the date of diagnosis until the date of death. Multivariate analyses were performed using stepwise logistic regression methodology.

RESULTS

One hundred ninety-eight patients were studied (111 male, 87 female; median age 57, range 7 to 85 years). Only three pediatric patients were included in this study. Six patients were not treated, and 120 of the 192 treated patients achieved CR for a CR rate of 63% (Table 2). Complete response to induction therapy varied with age, in particular deteriorating in patients aged >60 years. In patients aged <60 years, resistant leukemia was the major cause of induction failure, with only ~5% of patients dying because of failure of supportive care.26 Similarly, resistant leukemia accounted for more than half of the induction failures in patients aged >60 years. This effect of age on response rate was of borderline statistical significance (P = .053 by chi-square analysis).

The relationships among FAB, age, and CR rate are shown in Table 3. Patients with FAB M3 morphology (acute progranulocytic leukemia) and M4 EOS (myelomonocytic leukemia with eosinophilia), tended to be younger, with median ages of 32 and 42 years, respectively. The response rates were highest in these two groups, with no patient in either group failing to achieve remission because of chemotherapy-resistant leukemia. In contrast, only 14% of patients with what has been termed “minimally differentiated acute nonlymphocytic leukemia” (M0) achieved CR with all failures related to chemotherapy-resistant leukemia.22

Cytogenetic findings and some FAB classifications were closely associated (Table 1). All patients with FAB M3 had a t(15;17) whereas all patients with M4 EOS had abnormalities at chromosome band 16q22. Similarly, 12 of the 13 patients with t(8;21) were in the FAB M2 class. There was no other consistent morphologic association. Clonal abnormalities were identified in 69% of the 179 patients with marrow specimens adequate for cytogenetic analysis and in 65% of the overall group of patients in whom chromosomal analysis was attempted (Table 1); t(8;21), t(15;17) and abnormalities of chromosome 16q22 were found in 17% (31

| Table 1. Cytogenetics and Patient Age: Response to Therapy and Morphology |
|--------------------------|-----------------|-----------------|-----------------|
| Cytogenetics             | n (% of Patients)* | Median Age (yr) | CR % (n) FAB |
| t(8;21)                  | 13 (7)           | 32 (24-71)      | 82 (9/11) 12 M2 |
| t(15;17)                 | 9 (5)            | 32 (25-74)      | 78 (7/9) 9 M3   |
| t(9;22)                  | 2 (1)            | 57 (64-81)      | 50 (1/2) 1 M1, 1 M2 |
| abn 16q22                | 9 (5)            | 42 (7-63)       | 89 (8/9) 9 M4 EOS |
| abn 8q24                 | 1 (1)            | —                | 0 (0/1) |
| abn 11q23                | 5 (3)            | 67 (7-69)       | 40 (2/5) 3 M1, 1 M4, 1 M5 |
| 5q- or -5 and 7q- or -7  | 17 (9)           | 60 (35-79)      | 41 (7/17) Median age 61 |
| 5q-                      | 8 (4)            | 63 (37-81)      | 50 (4/8) CR 46% (17/37) |
| -5                      | 3 (2)            | 57 (52-67)      | 33 (3/3) 2M0, 9M1, 15M2 |
| 7q-                      | 3 (2)            | 65 (41-70)      | 100 (3/3) 5M4, 4M6, 2M7 |
| -7                      | 7 (4)            | 52 (22-76)      | 33 (2/6) |
| 6q-                      | 2 (1)            | 61 (60-62)      | 100 (2/2) 2 M1 |
| +8                      | 7 (4)            | 64 (45-75)      | 29 (2/7) 1 M0, 2 M1, 2 M4 |
| Pseudodiploid           | 15 (8)           | 48 (27-78)      | 100 (15/15) MISC |
| Hyperdiploid            | 14 (8)           | 50 (41-69)      | 46 (6/13) MISC |
| Hypodiploid             | 13 (7)           | 51 (19-68)      | 69 (8/13) MISC |
| Normal                  | 51 (28)          | 59 (18-85)      | 65 (32/49) MISC |
| Borderline normal       | 9 (5)            | 64 (34-69)      | 44 (4/9) MISC |
| No mitoses              | 10 (5)           | 50 (32-72)      | 60 (6/10) |

MISC, no particular pattern of morphology.

*Expressed as the percentage of the number of patients with adequate specimens (n = 188).
of 179) of these patients. These patients tended to be younger and their CR rate was high (24 of 29, 83%).

The abnormal 5 and/or 7 grouping included patients with findings of −5, 5q−, 7q−, −7, or combinations of these. Many of the abnormalities of 5q− or 7q− involved interstitial deletions, whereas other patients had unbalanced translocations which resulted in loss of part of 5q or 7q. The overall outcome of such patients was very similar, and in subsequent analyses, the data for these patients were pooled. Abnormalities of chromosome 5 and/or 7, trisomy 8, and abnormalities of chromosome 11 at band q23 were found in 28% of patients, tended to occur in older patients, and were associated with statistically significantly inferior CR rates. This effect was independent of age in the patient with younger age groups, the three CR duration is shown in aged >60 years (P = .002). Cr duration deteriorated in patients aged >60 years at of borderline significance. Patients with normal and abnormal karyotypes were virtually superimposable (data not shown). Similarly, there were no significant differences among the patients in the miscellaneous hypodiploid, pseudodiploid, and hyperdiploid cytogenetic categories; neither was there a difference within the different groups of patients with abnormalities of chromosome 5 and/or 7. Figure 3 shows that three definable clusters statistically significant from one another could be defined. Patients with abnormalities of 16q22 have the best overall prognosis, whereas patients with t(15;17) or t(8;21) have an intermediate prognosis. Patients with FAB M2 morphology and t(8;21) had a superior survival duration as compared with other patients with FAB M2 (median 31 vs 12 months, P = .025) despite similar CR induction rates (9 of 11 vs 36 of 52, P = NS). Last, patients with 11q23 abnormalities, −5/5q− and/or −7/7q−, or trisomy 8 have a very poor overall outcome with only a single long-term survivor.

CR duration curves for the same cytogenetic categories are shown in Fig 4. Again a statistically significant difference, identical to that seen for overall survival duration, existed between the groups with a poorer prognosis and the other groups. Although the number of patients was small, there was no difference in CR duration between patients with abnormal 16q22 and the t(15;17) or t(8;21) patients. FAB M2 patients and t(8;21) had somewhat longer CR durations as compared with other patients with FAB M2 morphology.
abnormalities who relapsed (median 26 v 13 months, \( P = .088 \)). Two patients with 16q22 abnormalities who relapsed have had unmaintained second remissions of >4 years, accounting for the survival advantage shown in Fig 3. The CR durations were virtually identical when patients with normal and abnormal karyotypes were compared with similar projected rates of long-term disease-free status. Last, the CR duration was 18 months in the pseudodiploid patients with the 100% CR rate, with 33% of patients in unmaintained CR at >48 months.

The results in patients with FAB-M3 and M4 EOS morphology correspond to the cytogenetic results described above. Patients with minimally differentiated (FAB M0), monocytic (FAB M5), and megakaryocytic leukemia (FAB M7) did very poorly overall; no patient remains alive and only one survived for 2 years. Three of the four patients with erythroleukemia (FAB-M6) who achieved CR had prolonged CR, with two patients remaining in unmaintained CR at 27+ and 62+ months (Fig 5). None of these four patients had abnormalities of chromosomes 5 or 7 or trisomy 8. Although the overall survival was superior in patients with FAB M2 as compared to FAB M1 morphology, this was accounted for entirely by differences in CR rate (Table 3) in that the CR duration (median 14 months) and the fraction of patients remaining in long-term CR (26% to 28% of CR patients) were identical in the two groups. With the exception of the M4 EOS patients (Fig 3), patients with myelomonocytic leukemia (FAB M4) experienced the longest CR durations (median 55 months), with five patients currently in CR at 60+ months (Fig 5).

Because of the considerable overlapping among the FAB, cytogenetic, and age groupings, a stepwise logistic regression analysis was done (Table 4). Age >60 years was the only significant independent prognostic factor adversely affecting achievement of CR (\( P = .01 \)) and overall survival (\( P = .004 \)). CR duration was adversely influenced by the cytogenetic findings of trisomy 8 (\( P = .002 \)) and abnormalities of chromosomes 5/7 (\( P = .01 \)). Consistent with the analyses described above, the presence of inv16 (equivalent to FAB M4 EOS) and t15;17 (similar to FAB M3) were major predictors of more prolonged survival and CR duration. Other cytogenetic findings were predictive of longer survival because of their effect on achievement of CR, but not on CR duration. FAB M4 morphology was the strongest predictor of longer CR and survival durations (Fig 5) owing largely to the five patients who remain in disease-free survival >60 months. Two of these patients had normal karyotypes; three had different pseudodiploid karyotypes including t(3;5), 2q−, and r18.

**DISCUSSION**

The overall results of this study confirm that a high percentage of patients with ANLL treated with intensive induction therapy achieve CR and indicate that a fraction of these patients obtain a prolonged disease-free survival with the likelihood that many of these patients will be cured of their disease. Disease-free survival is more readily achieved in younger patients although it is notable that the dropoff in CR rate and overall survival did not occur in this series except in patients aged >60 years. This is in part related to the improvements in supportive care that have occurred in the last decade.27

Generally when more elderly patients achieve CR remission, the duration of CR has been believed to be equivalent to
that of younger patients. Although the shorter CR duration in older patients was of borderline significance in the current study, other recent studies with de novo ANLL have also reported shorter CR durations in older patients. This observation probably is related to a potential different “biology” of the ANLL, in that the frequency of adverse cytogenetic findings, in particular trisomy 8 and abnormalities of chromosome 5 and/or 7, are considerably more frequent in older patients. Indeed, multivariate analysis indicated that abnormalities of these two chromosome groups were the only independent predictors of inferior CR duration. The somewhat higher incidence of abnormalities of 5 and/or 7 in our series may be a function of the high median age in our group of patients.

Deletions of all or part of chromosomes 5 and/or 7 have been described in patients with preleukemic syndromes, prior treatment with chemotherapy and/or radiotherapy, and suspected exposure to environmental mutagens. Patients who develop leukemia following such circumstances are known to have a poor prognosis, usually because of chemotherapy resistant disease. Recent studies of large numbers of patients in international registries also confirm the increased incidence of these cytogenetic findings in older patients, suggesting a cumulative effect of as yet undefined environmental exposures. The present series included only patients with apparent de novo leukemia defined clinically by the absence of a pancytopenic prodom or prior treatment. The similarity in cytogenetics, age distribution, and prognosis suggest that these de novo leukemias may have a common etiology with the “secondary” leukemias. Trilineage dysplasia is a common feature in patients with preleukemic syndromes and treatment-induced leukemias. This was not a consistent finding in our patients with abnormalities of chromosomes 5 or 7. Eight patients had distinct morphologic abnormalities in all cell series. Four of these patients had erythroleukemia (FAB M6) and two patients had additional abnormalities of chromosome 3 (inv(3)(q21q26) which have been associated with abnormal megakaryocytopenia, although perhaps not uniquely.

Trisomy 8 was also more common in older patients, as has also been described in recent large adult studies. Of note, however, is that trisomy 8 was the most common single cytogenetic finding recently noted in a large study reported by the Children’s Cancer Study Group. The CR group in this pediatric group was 94%, although long-term survival data were not provided. Samuels et al described a higher CR rate (60%) in adult patients with trisomy 8 than was observed in our patients, although only 9% of these patients remained in CR at 2 years. Further pediatric data are needed to determine whether trisomy 8 has the same negative impact in younger patients.

Conversely, certain cytogenetic findings confer a much better prognosis, particularly with respect to remission induction results. Although the detection of a pseudodiploid karyotype was the only independent predictor of achieving CR, a wide variety of different chromosomal changes was noted in this grouping. The median CR duration in the pseudodiploid patients was identical to that of the entire group of patients, further suggesting that this does not represent a unique subgroup. Patients with 16q22 abnormalities, t(8;21), and t(15;17) rarely failed to achieve remission because of resistant disease; ultimate failure is instead a consequence of later relapse. Patients with 16q22 abnormalities have a particularly good outlook; multivariate analysis confirmed the independent positive prognostic influence of this cytogenetic finding. Of some note is that two of our patients with 16q22 alterations have had prolonged unmaintained second remissions following first remissions of “average” length. Both second responses was achieved using diaziquone and further investigations of new drugs should consider focusing on the occasional long-term survivors of such approaches to determine whether certain cytogenetically definable subgroups might benefit from one type of therapy or another. This is particularly important because prolonged second remissions are being noted with increased frequency in subgroups of patients with ANLL. Indeed, a recent report has suggested that patients with inv (16)(p13q22) in particular may have sustained second remissions.

The long-term results in our 49 patients whose karyotypes appeared normal were similar to those of the “intermediate”-risk group, with median survival duration of 12 months and 20% long-term survivors and a median remission duration of 17 months, with 30% of complete responders projected to

### Table 4. Multivariate Analysis

<table>
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<th>Characteristics</th>
<th>CR %</th>
<th>P Value</th>
<th>Survival Duration</th>
<th>P Value</th>
<th>CR Duration</th>
<th>P Value</th>
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<td></td>
<td>Age &gt; 60 years</td>
<td>.0004</td>
<td>Trisomy 8</td>
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<td>FAB M4</td>
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<td>FAB M4</td>
<td>.0012</td>
<td>Abnormal 16q22*</td>
<td>.015</td>
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<tr>
<td>t(8;21)</td>
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<td></td>
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<td>FAB M2</td>
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<td>t(15;17)</td>
<td>.03</td>
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Only statistically significant factors (p < .05) are listed. The factors considered were age (divided into four discrete categories, pooling all patients age > 60 years—Table 1), and all cytogenetic and FAB categories where n > 5.

*Equivalent to FAB M4 EOS.
remain in long-term remission at 48 months. Some of these patients may have had subtle structural rearrangements which remain undetectable with current technology, and future studies using potentially available new molecular probes may also detect subgroups within this classification of patients. Similar results were also noted in patients with miscellaneous pseudodiploid abnormalities. Larger studies, such as those being conducted by the Cancer and Acute Leukemia Group B,49 are needed to define the possible impact of specific abnormalities within this group.

Somewhat surprisingly, particularly in view of the very poor survival noted in patients with monocytic leukemia (FAB M5), myelomonocytic histology (FAB M4) was the strongest independent factor associated with more prolonged CR duration and survival. There were no particular cytogenetic findings in these patients. Most large chemotherapeutic trials have not reported their results according to FAB classifications, have pooled FAB M4, FAB M4E0S, and M5 patients, or have included patients with secondary leukemia. Mertelsmann et al noted an 88% CR rate in 18 patients with M4 histology, although the median survival was 23 months.3 These authors, as well as other groups,2,40,41 have reported that FAB M4 can be difficult to categorize reliably with considerable interobserver variability. Although our 28 patients were classified according to updated FAB criteria using appropriate histochemical staining,2 analyses of larger series of patients would be advisable before one concludes that FAB M4 confers an unusually favorable outlook.

With currently available cytogenetic techniques, ~40% to 50% of patients with ANLL can be categorized into discrete subgroups with differing prognoses. The identification of such groupings suggests that different therapeutic modalities might be used in such patients in the future. Although use of either allogeneic or autologous bone marrow transplantation in patients with ANLL in first remission is currently of interest,42,43 it will probably not be a fruitful approach in patients who have abnormalities of chromosomes 5 or 7 or trisomy 8. Even for patients who enter remission, bone marrow transplantation is generally not an option because of their more advanced age. Whether transplantation would necessarily be more effective is also unclear because recent evidence shows that the relapse rate posttransplant may also be higher in patients with “poorer prognosis” leukemia identified by other means, such as monocytic histology or initially elevated WBC count.44 Furthermore, the remission rate in such patients is low, usually related to drug-resistant disease, suggesting that different forms of induction therapy should be considered in such patients. Response rates in such patients may not be improved appreciably by further manipulations of anthracycline and Ara-C combinations, and use of newer agents during induction should be considered. Many of these patients can be identified because of gross chromosomal deletions or additions which should be detectable within a reasonably short period of time (<2 weeks) after the diagnosis of ANLL is made. Because treatment must often be given immediately after diagnosis to patients with ANLL, one might consider protocols in which therapy is modified at days 7 to 10 based on detection of certain cytogenetic findings. Newer molecular techniques could make routine detection of chromosomal gains or losses more rapid in the future.

In contrast, patients with “better prognosis” karyotypes have a very good chance of entering remission but have an appreciable rate of relapse and eventually develop chemotherapy-resistant disease. In this group of patients, most of whom have balanced translocations, bone marrow transplant or other intensive postremission approaches, perhaps using new chemotherapeutic agents, would be worthy of consideration. In such patients, cytogenetic results available 3 to 4 weeks after diagnosis could serve as guidelines for differing therapeutic options after remission is achieved.

The development of resistant disease, even in patients with better prognosis suggests that serial cytogenetic studies would be of interest in an attempt to detect the emergence of chemotherapy-resistant clones. The current Chicago classification of cytogenetic abnormalities characterizes patients according to the “dominant” finding.18 Detection of sublines, at the time of diagnosis, with additional cytogenetic abnormalities or subsequent development of such clones is not uncommon, however.41 For example, patients with t(15;17) also commonly have an additional chromosome 8.12,16 From the limited data available, whether such patients have a poorer prognosis than do patients with t(15;17) alone is not clear. Additional chromosomal changes can also occur when patients are followed serially.45 Larger studies than the current one are necessary to determine the impact of such findings on long-term response to therapy, and such studies are of great importance because they may help to characterize further additional subgroups of patients for whom different therapeutic approaches may be individually tailored. In addition, they may allow experiments that assess the mechanism by which cytogenetic changes appear to be associated with both alterations in normal differentiation and leukemogenesis and ultimate resistance to therapy.

Indeed, this aspect of the impact of cytogenetics on ultimate prognosis is probably the most exciting. The specific chromosomal changes probably result in either the production or the absence of specific gene products which result in the abnormal halt in differentiation characteristic of acute leukemia. The tight association with particular morphologic subtypes provides support for this contention. In addition, because of the influence on prognosis, further characterization of the specific gene products should eventually result in more precise determinations of the mechanisms by which cells become resistant to therapy and suggest interventions to circumvent this resistance. For example, the gene coding for the p-glycoprotein associated with the multidrug-resistance phenotype was recently mapped to chromosome 7q (46), suggesting that parallel studies of expression of this gene in patients with specific cytogenetic findings would be of considerable interest.

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