Prognostic Importance of Mutations in the ras Proto-Oncogenes in De Novo Acute Myeloid Leukemia


Mutations of the N- and K-ras genes are the most frequent genetic aberrations in acute myeloid leukemia (AML) and their detection in preleukemic conditions such as the myelodysplastic syndrome (MDS) suggests a role in the earliest phases of leukemogenesis. Despite these observations, little is known about the clinical importance of ras mutations in AML. We studied the clinical impact of ras mutations in 99 patients with de novo AML. All patients were treated in two prospective multicenter trials. The polymerase chain reaction was used to amplify areas surrounding the codons 12, 13, and 61 of the three ras genes N-, K-, and H-ras from DNA from bone marrow cells. ras mutations were detected by an algorithm based on allele-specific oligonucleotide hybridization. Eighteen of 99 (19%) patients harbored mutations in either N- or K-ras. All of the observed mutations occurred in N-ras (N = 10) and K-ras (N = 5) or concurrently in both N- and K-ras (N = 3). There were no significant differences between ras-negative and ras-positive patients according to age, sex, blood counts, cytogenetic abnormalities, or French-American-British classification. However, univariate analysis suggested a longer survival in ras-positive patients (P = .11). When adjusted for age, which was the most important factor affecting outcome, the presence of a ras mutation emerged as a significant predictor for improved survival (P = .03) along with lower bone marrow blast counts (P = .02) and better cytogenetic category (P = .01). However, the presence of an aberrant ras allele was strongly correlated with lower bone marrow blast counts (P = .007). Thus, whether a mutation in the N-ras or K-ras proto-oncogenes directly affects treatment outcome or indirectly through an association with lower leukemic burden remains to be determined. Nevertheless, these findings counter the prevailing bias that oncogene mutations lead to more aggressive behavior in human malignancies. © 1994 by The American Society of Hematology.

MEMBERS OF THE ras proto-oncogenes have been implicated in a variety of human malignancies and are among the most frequent genetic aberrations detected in human tumors. The three ras genes commonly involved, N-, K-, and H-ras, encode for closely related proteins, p21ras, which localize to the inner plasma membrane and have intrinsic GTPase activity. Mutations in critical regions of the p21ras gene attenuate this GTPase activity, activate the oncprotein, and lead to oncogenic transformation.

With respect to hematologic malignancies, ras proto-oncogene mutations are the most commonly observed molecular alteration, with the reported incidence ranging from 20% to 30% in acute myeloid leukemia (AML). In AML and in the myelodysplastic syndromes (MDS), ras mutations have been associated with monocytic differentiation. Studies in MDS, furthermore, suggest that ras mutations may encode for a higher proliferative activity of the affected clone. Data imply that biologic differences might be expected between patients with and without a ras mutation in de novo AML and that these biologic differences may lead to differences in response to chemotherapy and, potentially, to patient survival.

We sought to assess the role of ras proto-oncogene mutations with respect to their biologic and clinical relevance in adult AML. Using the polymerase chain reaction (PCR), we amplified ras sequences surrounding the critical codons 12, 13, and 61 in N-, K-, and H-ras from 99 patients with de novo AML enrolled in a national cooperative group's treatment studies. The mutations were then detected using oligonucleotides specific for the individual mutations with a sensitive PCR-based technique. Analysis of survival data shows that mutations in the ras proto-oncogenes may portend a favorable prognosis in de novo AML.

MATERIALS AND METHODS

Patients. The 99 patients in this study were recruited from the Cancer and Leukemia Group B (CALGB) treatment studies number 8525 (N = 86) and 8821 (N = 13). The genetic analyses were performed as part of CALGB companion protocols 8361 and 8765, which procured blood and bone marrow samples prospectively on patients entered on CALGB treatment studies. The diagnosis of AML was made with standard bone marrow and blood smears according to the French-American-British (FAB) classification. All slides were centrally reviewed. Patients with a history of myelodysplasia or having previously received chemotherapy were excluded from these studies. In 2 patients, the exact lineage of the leukemia could not be ascertained on the initial morphologic examination.

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however, in both cases, cytogenetic and immunophenotyping data supported the diagnosis of a myeloid leukemia. In 1 case, cytogenetics [the presence of t(9;11)] and immunophenotyping (CD11b+, CD14+, CD15+, and CD33+ staining) were consistent with AML. In the second case, the immunophenotyping (CD13+, CD14+, CD15+, and CD33+ staining) was consistent with a myeloid leukemia. Thus, these patients were grouped as AML, unclassified (MO-AML).

Cytogenetics were performed in CALGB-approved institutional laboratories as part of a prognostic karyotype study. CALGB 8461, and were centrally reviewed. Sixty-seven of the 99 patients had adequate cytogenetics and, of these, 30 cases (44%) had normal karyotypes and 56% were abnormal. In the overall group of 1,272 cases enrolled in the CALGB cytogenetics study, 56% of adequate cases were also abnormal. Thus, our cytogenetic distribution was representative of the larger group experience.

Previous CALGB experience has shown that patients with t(8;21), abnormalities of 16q, t(15;17), and patients with normal cytogenetics have a favorable prognosis as compared with those with other specific karyotypic abnormalities.12 Thus, for these present study, patients were categorized into a priori cytogenetic groups: better [including t(8;21), abnormalities of 16q, t(15;17), and normal cytogenetics] and worse (all other karyotypic changes).

CALGB 852513 was a treatment protocol in which induction chemotherapy consisted of daunorubicin (45 mg/m2 for 3 days [30 mg/m2 in patients >60 years of age]) and cytarabine (200 mg/m2 for 7 days). Patients who did not achieve complete remissions (CR) were removed from protocol therapy (N = 33). Patients in CR (N = 53) were then randomized into three arms: cytarabine 3 g/m2 for 6 doses (high dose); 400 mg/m2 for 5 days by continuous infusion (intermediate dose); or 100 mg/m2 for 5 days (low dose). Fourteen patients received high-dose cytarabine (N = 10, ras positive; N = 4, ras negative). 19 patients intermediate-dose cytarabine (N = 17, ras negative; N = 2, ras positive), and 20 patients low-dose cytarabine (N = 15, ras negative; N = 5, ras positive). No statistically significant differences were noted in the distribution of ras-positive and ras-negative cases among the various cytarabine arms (P = .38). This postremission chemotherapy program was repeated every 4 weeks for 4 months. After the initial 4 months of treatment, all patients received cytarabine (200 mg/m2 on 5 consecutive days) and daunorubicin (45 mg/m2) for another four cycles. Thereafter, the patients were follow-up without further therapy. CALGB 8821 was a study in which cytarabine (200 mg/m2 for 7 days) was combined with daunorubicin (45 mg/m2 for 3 days) as induction and mitoxantrone/diazaquone, and etoposide/cyclophosphamide were then successively administered in two intensification courses.14 Nine patients treated as part of CALGB 8821 were ras positive and 4 were ras negative. In both CALGB studies, the age requirement for eligibility was greater than 13 years of age and no upper age limit was imposed. The distribution of the ras-positive and ras-negative cases did not differ amongst the various treatment groups (P = .42).

The median survival for all 99 patients was 1.2 years, with a median follow-up time of 4 years. No difference in survival was noted between patients entered on CALGB 8525 or 8821 (P = .25).

Blood and bone marrow cells were isolated on a Ficoll gradient. High molecular weight DNA was isolated using standard procedures15 and the molecular analysis was performed in a blinded fashion. Patient samples were identified randomly from the larger study group.

PCR. PCR was performed with 200 ng DNA under standard conditions in 100 μL (50 mMol/L KCl, 10 mMol/L Tris CI, pH 8.3, 1.5 mMol/L MgCl2, 0.001% [wt/vol] gelatin [Sigma, St Louis, MO], 200 μMol/L dNTPs [Boehringer Mannheim, Mannheim, Germany], and 2.5 U AmpliTaq [Perkin Elmer Cetus, Norwalk, CT]) in a Thermocycler (Perkin Elmer Cetus). Sequences surrounding N-, K-, and H-ras codons 12, 13, and 61 were amplified using the following protocol: cycle 1, 94°C for 5 minutes, 55°C for 1 minute, and 72°C for 1 minute; cycles 2 through 35, 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. The oligonucleotide primers were used at 0.5 μMol/L and purchased from Clontech (Palo Alto, CA). In the first screen of the samples, PCR-amplified DNA was slot-blotted and probed with a mixture of up to six mutational specific oligonucleotides (Clontech) as described (probes for a total of 57 possible mutations were applied in each patient).11 Each sample was investigated at least twice. After completing the molecular analysis on all 99 samples, the genomic DNA of positive samples was subjected to a second PCR amplification. Seven microilers of the PCR amplification was electrophoresed on a 3% NuSieve (FMC, Rockland, ME)/1% agarose gel and hybridized with individual mutational specific probes after Southern transfer, as previously described.11 This allele-specific oligonucleotide hybridization (ASOH) approach is sufficiently sensitive to detect one mutant allele in the presence of nine wild-type ras genes.10 Selected cases harboring ras mutations were subjected to further scrutiny by analyzing the peripheral blood DNA for the presence of the same mutation that was found in the bone marrow. In every case, the identical ras lesion was detected in both tissue sources. Four samples with ambiguous hybridization results were subjected to direct sequencing of the PCR products for sequence confirmation and, in each case, the sequence results corroborated the ASOH data.

Analysis of clinical data. The molecular analyses were performed on the coded samples in a blinded fashion; the data from this analysis were then examined for association with the clinical parameters. Two end-points were used in the analysis: (1) survival time as defined by the time from registration on study to the date of death from any cause (patients still alive were censored at the date of last contact), and (2) remission duration as measured from the date of CR to relapse or death. Patients still in continuous CR were censored at date of last follow-up.

Statistical methods. The following pretreatment patient characteristics were compared between the group of patients with a ras mutation and the group of patients without such mutations: age, sex, peripheral white blood cell count (WBC), platelet count, percentage of blasts in bone marrow, FAB classification, and cytogenetic classification [better: t(8;21), t(15;17), normal karyotype]. Analysis of frequencies was performed using the Fischer exact test. Comparisons of survival curves were performed using the log-rank statistic.17 The prognostic significance of the study variables, after adjusting for age, was assessed using the Cox proportional hazards model.18 Specifically, each study variable was considered individually in the presence of age, and the resulting increase in log-likelihood was tested for each variable by the likelihood ratio test. The natural logarithm of the WBC count and platelet count, denoted log[WBC] and log[platelets], respectively, were used to adjust for skewness in the distribution of these variables. Hazard estimates (and 95% confidence intervals) were obtained from the Cox model by exponentiating the regression coefficients.
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Fig 1. Slot-blot analysis of N-ras codon 12 on 30 patient samples (see Materials and Methods for details of the analysis). (A) Filter hybridized with an oligonucleotide probe specific for the wild-type N-ras sequence in codon 12 showing all samples harboring the wild-type allele. (B) Filter hybridized with a mixture of oligonucleotide probes specific for all possible single-base mutations in codon 12 showing two samples with putative mutations.

parameter at presentation was associated with the presence of a ras mutation: specifically, age (median age of ras-positive group was 55.3 years and of the ras-negative group was 48.8 years; \( P = .82 \)), FAB classification, cytogenetic grouping, blood counts, and sex (Table 1). Three ras-positive patients failed to respond to induction chemotherapy and, of the 15 ras-positive patients who achieved a CR (83% of 18 ras-positive patients), 4 remain in continuous CR. In the ras-negative group, 50 of 81 patients achieved CR (62%) and 19 patients remain in continuous CR.

Analysis of prognostic factors. The median survival of our entire cohort of 99 patients was 1.2 years. Univariate Cox regression analysis identified age as the most significant factor associated with survival duration (\( P < .001 \); Table 2). The median survival of patients under 40 years of age was 21 months, as compared with 12 months for patients between 40 and 59 years, and 3 months for those over 60 years of age. An increased peripheral leukocyte count was also associated with a poorer prognosis (\( P = .02 \)), as was a high platelet count at diagnosis (\( P = .04 \)). Interestingly, the presence of a mutant ras proto-oncogene appeared to be associated with a more favorable outcome, although the association was not statistically significant (Table 2). The median survival of AML patients without ras mutations was 13 months, whereas that of patients with mutant ras genes was 19 months (log-rank test, \( P = .13 \); Fig 3A). No factor other than age, the WBC count, and the platelet count had a statistically significant association with survival when considered individually.

Because the most significant prognostic variable, age, may interact and obscure the impact of other factors on outcome, we sought to normalize the effect of age on the predictive value of the other four study variables using a multivariate Cox regression analysis. When age was taken into
account, the presence of a ras mutation emerged as a significant predictor for better survival \((P = .03)\), as did the cytogenetic status \((P = .01)\) and the percentage of the bone marrow replaced by leukemic blasts \((P = .02, Table 3)\). In this analysis, the hazard ratio is an estimate of the proportional increase in risk of death at any given time and age for a patient in a "worse" category relative to a patient in a "better" category. For patients without a ras mutation, the hazard ratio was 1.94 (95% confidence interval [CI], 1.01 to 3.76), indicating that their instantaneous risk of death is approximately twice that of patients of the same age harboring a ras mutation. However, the peripheral WBC count and the platelet count no longer affected survival \((P = .11\) and .18, respectively).

A better cytogenetic status and a lower percentage of blasts in the bone marrow were the only other factors that, after adjusting for age, remained associated with improved survival \((P = .01\) and .02, respectively; Table 3). Because the percentage of blasts in the bone marrow was statistically associated with the ras status (Table 1), we sought to determine the relative importance of the bone marrow blast count and the ras status in predicting survival outcome using multivariate analysis. After adjusting for age and per-

![Fig 2. PCR-Southern blot analysis for N-ras codon 12 mutations. Eight patient samples were amplified, transferred in six parallel lanes to nylon filter, and probed with the individual mutational-specific oligonucleotides ([A] through [E], see below) or the wild-type (F). The common sequences for the sequence-specific oligonucleotides for N-ras codon 12 are GGA GCT - GGC GTA GGC AA. The underlined sequence is (A) GAT coding for aspartate; (E) - GCT coding for alanine; (C) coding for cystine; (D) coding for serine; (E) GTT coding for valine; (F) coding for glycine (wild-type N-ras sequence). The mutations shown are two GAT mutations (lanes 6 and 8) and three AGT mutations (lanes 3, 5, and 7).](image)

| Table 1. Characteristics of the 99 Patients Relating to Clinical Data and ras-Mutational Analysis |
|-----------------|----------|----------|---------|
| ras Mutation    | N         | Present  | Absent  |
| All patients    | 99        | 18 (18)  | 81 (82) |
| Age (yr)        |           |          |         |
| Under 40        | 36        | 6 (17)   | 30 (83) |
| 40-59           | 38        | 8 (21)   | 30 (79) |
| 60+             | 25        | 4 (16)   | 21 (84) |
| Sex             |           |          |         |
| Male            | 47        | 6 (13)   | 41 (87) |
| Female          | 52        | 12 (23)  | 40 (77) |
| WBC count       |           |          |         |
| <20,000/µL      | 29        | 5 (17)   | 24 (83) |
| 20,000-50,000/µL| 24        | 4 (17)   | 20 (83) |
| >50,000/µL      | 46        | 9 (20)   | 37 (80) |
| Platelets       |           |          |         |
| <50,000/µL      | 50        | 8 (16)   | 42 (84) |
| 50,000-99,000/µL| 26        | 5 (19)   | 21 (61) |
| >100,000/µL     | 23        | 5 (22)   | 18 (78) |
| Percentage of bone marrow blasts | |         |         |
| <60             | 24        | 9 (37)   | 15 (63) |
| 60-79           | 30        | 6 (20)   | 24 (80) |
| 80-100          | 44        | 3 (7)    | 41 (93) |
| FAB classification |       |          |         |
| M1              | 2         | 14       | .42†    |
| M2              | 7         | 24       |         |
| M2E             | 0         | 1        |         |
| M3              | 0         | 7        |         |
| M3V             | 0         | 1        |         |
| M4              | 5         | 15       |         |
| M4E             | 0         | 1        |         |
| M6A             | 2         | 4        |         |
| M6B             | 1         | 7        |         |
| M6              | 1         | 0        |         |
| AML unclassified | 0        | 7        |         |
| Cytogenetics    |           |          |         |
| Better          | 42        | 11 (26)  | 31 (74) |
| Worse           | 26        | 3 (12)   | 23 (88) |
| Not evaluable   | 31        | 4 (13)   | 27 (87) |

The pretreatment values are shown. Percentages are in parentheses. * Pearson χ² test; 1 patient did not have complete bone marrow biopsy data but showed 38% blasts that contained Auer rods and 48% promyelocytes in the peripheral blood. This patient was assigned as AML unclassified.
† Fisher two-tailed exact test, better versus worse categories.
‡ M4 and M5 (B ras positive/35) versus other FAB classifications (10 ras positive/64).
approximately 25% of cases. Variations in ras the most common molecular abnormality

class associations could be better refined by examining the

tations in codon changed. In this analysis, a significant age difference was ob-

erved between patients with mutations in codons 12, 61, 7. The most frequent base transition was a G to T

uation status; however, our analysis did not show a prognostic value for the ras mutation status independent of bone marrow blast percentage, larger numbers of patients will be needed to resolve this question.

In seeking explanations for the improved survival of the ras-positive cases, we examined those parameters that may impact on overall survival. Neither complete remission rate (ras positive [83%] vs ras negative [62%], P = .10), event-free survival (P = .20), duration of complete remission (P = .88, Fig 3B), nor survival after relapse (P = .39) was statistically different between the two ras groups.

Associations with specific ras mutations. Of the 18 ras-positive cases, 21 specific ras mutations were detected: 8 were in N-ras codon 12, 1 in N-ras codon 13, 4 in N-ras codon 61, 7 in K-ras codon 12, and 1 in K-ras codon 61 (Table 4). The most frequent base transition was a G to T exchange at codons 12 and 13 of N- and K-ras accounting for 13 of the 21 ras mutations. Analysis of the clinical factors associated with the specific ras mutations showed that, although patients with G to T transitions seemed to be older than those with other base changes, this difference was not statistically significant (excluding patient no. 69 with both types of base substitutions; median age, 55 years v 33 years, P = .13, Mann-Whitney test). Because all G to T changes occurred in codons 12 and 13, but not all codon 12 or 13 mutations were G to T mutations, we asked whether clinical associations could be better refined by examining the specific codons affected rather than the specific bases changed. In this analysis, a significant age difference was observed between patients with mutations in codon 12 or 13 (median age, 55.8 years) as compared with those with mutations in codon 61 (median age, 24.6; P = .02, Mann-Whitney test).

DISCUSSION

Mutations in the ras proto-oncogenes have been implicated in the genesis of AML and have been described in approximately 25% of cases. This frequency makes aberrations in ras the most common molecular abnormality in this heterogeneous disease. Despite the prevalence of ras mutations, the clinical significance of such molecular aberrations in AML is unclear. To assess the impact of ras mutations on this disease, we sought to analyze a relatively uniform cohort of de novo AML patients who were diagnosed in a standard fashion and treated with intensive chemotherapeutic regimens.

In this study, we investigated the frequency and significance of ras mutations in 99 adult AML patients enrolled in two CALGB treatment protocols. The frequency of ras mutations in this prospective trial is in keeping with similar studies: of 99 patients (18%) harbored a aberrant ras alleles with 62% of these mutations in N-ras and 38% in K-ras. We show that, after adjusting for age, the presence of any ras mutation appears to be a significant predictor of improved survival in AML. Other parameters previously shown to affect survival such as the percentage of blasts in the marrow and cytogenetic status also had impact on the clinical outcome. The cytogenetic status was not associated with the ras mutation status; however, patients with bone marrow blast content of less than 60% were more likely to be ras positive (P = .007). Multivariate analysis did not show that the ras mutation status significantly contributed to survival in addition to the percentage of blasts in the marrow and to age in this patient cohort (P = .09). Thus, the survival advantage in the ras mutation-positive cases may be caused by the lower leukemia burden treated. Moreover, although the median follow-up time of our study (4 years) is considerable, it remains possible that further late relapses in the ras-positive group will narrow the differences between the ras-positive and -negative populations. Thus, longer follow-up and larger patient numbers will be required to resolve these issues.

Many factors may contribute to the favorable outcome associated with ras mutations; however, our analysis did not uncover any specific factor that may explain this result. Nevertheless, although the CR rate was not significantly different between those with and without ras mutations, the CR rate in the ras-positive group was greater than that of the ras-negative group (83% v 62%, P = .10). A similar finding was observed in an earlier study on 55 patients with de novo AML in that the CR rate in ras-positive patients was 63% versus 43% in ras-negative patients, leading to the specula-

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Table 2. Results of the Univariate Analysis With Respect to Survival Time

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Categories</th>
<th>Coefficient</th>
<th>x²</th>
<th>P Value</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>99</td>
<td>Continuous</td>
<td>0.037</td>
<td>21.44</td>
<td>&lt;.001</td>
<td>1.04 (1.02-1.05)</td>
</tr>
<tr>
<td>log(wbc)</td>
<td>99</td>
<td>Continuous</td>
<td>0.218</td>
<td>5.04</td>
<td>.02</td>
<td>1.24 (1.01-1.52)</td>
</tr>
<tr>
<td>log(platelets)</td>
<td>99</td>
<td>Continuous</td>
<td>0.285</td>
<td>4.06</td>
<td>.04</td>
<td>1.33 (1.01-1.78)</td>
</tr>
<tr>
<td>ras mutation</td>
<td>99</td>
<td>Present, absent</td>
<td>0.490</td>
<td>2.50</td>
<td>.11</td>
<td>1.63 (0.85-3.14)</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>68</td>
<td>Better, worse</td>
<td>0.381</td>
<td>1.51</td>
<td>.22</td>
<td>1.46 (0.81-2.63)</td>
</tr>
<tr>
<td>Percentage of blasts in bone marrow</td>
<td>98</td>
<td>&lt;60%, &gt;60%</td>
<td>0.368</td>
<td>1.73</td>
<td>.19</td>
<td>1.46 (0.81-2.50)</td>
</tr>
<tr>
<td>FAB</td>
<td>99</td>
<td>M4 and M5, others</td>
<td>0.106</td>
<td>0.19</td>
<td>.67</td>
<td>1.11 (0.70-1.74)</td>
</tr>
<tr>
<td>Sex</td>
<td>99</td>
<td>Females, males</td>
<td>&lt;0.001</td>
<td>&lt;0.00</td>
<td>&gt;.99</td>
<td>1.00 (0.63-1.60)</td>
</tr>
</tbody>
</table>

Variables significantly associated with survival are bolded.

* Positive coefficients indicate better survival for the lower values of the continuous variables (eg, younger age is associated with better survival) and for the first category listed for the dichotomous variables (eg, presence of ras mutation is associated with better survival).

† For continuous variables, the hazard ratio indicates the relative increase in the risk of death for a unit change in the variable (eg, 1 year, 1 log wbc).
tion that patients harboring aberrant ras alleles may potentially exhibit a better response to intensive antileukemic therapy. Supporting this notion, 6 of 10 previously reported cases with ras-positive AML relapsed with ras-negative leukemic clones, indicating that intensive chemotherapy can eradicate leukemic cells harboring an abnormal ras oncogene.

In earlier studies in which ras mutations and clinical outcome in adult AML have been analyzed, no statistically significant association between the presence of a ras mutation and survival had been observed. However, the results of these studies were based on small numbers of patients, thus precluding multivariate analysis. Furthermore, they were confounded by either incomplete ras analysis (eg, only the N-ras gene was assessed); by nonuniform treatment regimens; or by including patients with AML evolving from antecedent hematologic malignancies. Our study differs in the extent of the ras analysis and the fact that all patients had de novo AML. Other studies focusing on the clinical implication of ras mutations in lung adenocarcinomas and childhood acute lymphoblastic leukemias have found that patients harboring ras mutations were more likely to have a poor outcome compared with patients without ras mutations. The discrep-
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Table 3. Age-Adjusted Cox Regression Analysis With Respect to Survival Time

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Category</th>
<th>Coefficient*</th>
<th>Incremental ( x^2 )</th>
<th>( P ) Value</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetics</td>
<td>68</td>
<td>Better/worse</td>
<td>0.790</td>
<td>6.47</td>
<td>.01</td>
<td>2.20 (1.22-3.99)</td>
</tr>
<tr>
<td>Percentage of blasts in bone marrow</td>
<td>98</td>
<td>&lt;60%/&gt;60%</td>
<td>0.653</td>
<td>5.39</td>
<td>.02</td>
<td>1.92 (1.06-3.48)</td>
</tr>
<tr>
<td>ras mutation</td>
<td>99</td>
<td>Present/absent</td>
<td>0.665</td>
<td>4.68</td>
<td>.03</td>
<td>1.94 (1.01-3.76)</td>
</tr>
<tr>
<td>log [wbc]</td>
<td>99</td>
<td>Continuous</td>
<td>0.163</td>
<td>2.59</td>
<td>.11</td>
<td>1.18 (0.96-1.44)</td>
</tr>
<tr>
<td>log [platelets]</td>
<td>99</td>
<td>Continuous</td>
<td>0.183</td>
<td>1.77</td>
<td>.18</td>
<td>1.20 (0.91-1.58)</td>
</tr>
<tr>
<td>FAB</td>
<td>99</td>
<td>M4 and M5, others</td>
<td>0.176</td>
<td>0.50</td>
<td>.48</td>
<td>1.19 (0.65-1.38)</td>
</tr>
<tr>
<td>Sex</td>
<td>99</td>
<td>Females, males</td>
<td>0.026</td>
<td>0.01</td>
<td>.91</td>
<td>1.03 (0.77-1.23)</td>
</tr>
</tbody>
</table>

Variables significantly associated with survival are bolded.

* Positive coefficients indicate better survival for the first category listed for the dichotomous variables (eg, presence of <60% blasts is associated with better survival) and for the lower values of the continuous variables. Those categories significantly associated with better prognosis are underlined.

ancy between the ras effect in AML and these other malignancies may be caused by the innate biologic differences between the disease states. In experimental systems, a mutant ras allele can induce such disparate responses as transformation,\(^2\) differentiation,\(^2\) or maturation\(^2\) depending on the cellular context in which it is expressed. Furthermore, lung cancers, AML, and acute lymphocytic leukemia (ALL) are treated with different modalities and with different chemotherapeutic agents that may variably interact with a mutant ras protein. Thus, the impact of aberrant ras genes on clinical outcome is likely to be unique to the individual malignancy. In apparent contradiction to our findings, murine fibroblast (3T3) cells transfected with an activated ras gene have been reported to be more resistant to anthracyclines because of the augmented expression of the multidrug-resistance protein, MDR1.\(^2\) However, this induction of anthracycline resistance was not seen by others in the identical system.\(^2\) Furthermore, the response of hematopoietic systems to the action of various oncoproteins are frequently different from that of NIH3T3 cells, as is seen in the disparate phenotypes of leukemic cell lines as compared with murine fibroblast cell lines when transfected with HER-2/neu.\(^2\) Thus, any extrapolation of findings in 3T3 cells to primary leukemic cells should be performed with considerable caution.

Several unexpected findings emerged from our study. A significant association was discovered between the presence of a ras mutation and a lower fraction of blasts in the bone marrow. Currently, we have no biologic explanation for this result. Nevertheless, this observation suggests that mutant ras-bearing leukemic cells may harbor different clinical properties. Furthermore, our analysis of the clinical factors associated with the specific ras mutations showed that patients with codons 12 and 13 were significantly older than those with codon 61 changes (median age, 56 years v 25 years). The frequency of specific base substitutions and their location may reflect exposure to certain mutagens. In ani-

Table 4. Clinical and Individual Data on the ras-Positive De Novo AML Patients

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>Sex/Age</th>
<th>FAB</th>
<th>Codon</th>
<th>AA Wild-Type</th>
<th>ras Analysis AA</th>
<th>Mutation</th>
<th>Response</th>
<th>Survival Time (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>M/59</td>
<td>M4</td>
<td>N61</td>
<td>GLN</td>
<td>K12</td>
<td>GLY</td>
<td>ARG</td>
<td>1,603*</td>
</tr>
<tr>
<td>73</td>
<td>F/57</td>
<td>M4</td>
<td>N12</td>
<td>GLY</td>
<td>K12</td>
<td>GLY</td>
<td>ASP</td>
<td>1,687*</td>
</tr>
<tr>
<td>89</td>
<td>F/35</td>
<td>M5A</td>
<td>N61</td>
<td>GLN</td>
<td>K12</td>
<td>GLY</td>
<td>ASP</td>
<td>1,652*</td>
</tr>
<tr>
<td>177</td>
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<td>M4</td>
<td>N81</td>
<td>GLN</td>
<td>K61</td>
<td>GLYN</td>
<td>LYS</td>
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<tr>
<td>179</td>
<td>M/55</td>
<td>M2</td>
<td>K12</td>
<td>GLY</td>
<td>K12</td>
<td>GLY</td>
<td>ASP</td>
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<tr>
<td>298</td>
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<td>GLY</td>
<td>M12</td>
<td>GLY</td>
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<tr>
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<tr>
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<td>ASP</td>
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<tr>
<td>329</td>
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<td>GLY</td>
<td>N61</td>
<td>GLN</td>
<td>ALA</td>
<td>1,305</td>
</tr>
<tr>
<td>333</td>
<td>F/55</td>
<td>M2</td>
<td>K12</td>
<td>GLY</td>
<td>N61</td>
<td>GLN</td>
<td>ALA</td>
<td>1,299*</td>
</tr>
<tr>
<td>344</td>
<td>F/21</td>
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<td>GLN</td>
<td>ASP</td>
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<tr>
<td>356</td>
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<td>M5B</td>
<td>N12</td>
<td>GLY</td>
<td>N12</td>
<td>GLN</td>
<td>ASP</td>
<td>1,205</td>
</tr>
</tbody>
</table>

Abbreviations: AA, amino acid; CR, complete remission; CR, Rel, CR, then relapsed; NR, no response.

* The patient was alive at last follow-up.
minal models, carcinogens such as methyl-nitrosourea and dimethylbenzanthracene induce specific ras mutations and can direct these mutations to either codon 12 or 61.\textsuperscript{39,30} Whether the association between age and the type of ras mutation is caused by specific exposure to different carcinogens or to differences in mechanisms of DNA repair is currently unclear.

Established prognostic indicators in de novo AML include age,\textsuperscript{31} cytogenetic abnormalities,\textsuperscript{32} and the presence of dysmyeloipoiesis.\textsuperscript{32} With regard to genetic abnormalities, there are some cytogenetic aberrations that correlate with a poor (eg, trisomy 8, abnormal chromosome 5 or 7) and with a favorable prognosis [eg, t(15;17), t(8;21), abnormalities of 16q22, and normal karyotype]. Our analysis suggests that the presence of a mutation in ras portends a favorable prognosis in de novo acute myeloid leukemia, but that this improvement may be caused by a decreased leukemia burden. The relative importance of ras mutations and bone marrow blast counts in determining treatment outcome in AML will require an analysis of larger numbers of patients.

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REFERENCES

Prognostic importance of mutations in the ras proto-oncogenes in de novo acute myeloid leukemia

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