Activating Mutations of N- and K-ras in Multiple Myeloma Show Different Clinical Associations: Analysis of the Eastern Cooperative Oncology Group Phase III Trial

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Mutations of members of the ras family are among the most common oncogene mutations found in multiple myeloma (MM). We have examined the mutational status of the N- and K-ras genes at codons 12, 13, and 61 in 160 newly diagnosed MM patients enrolled on the Eastern Cooperative Oncology Group (ECOG) phase III trial E9486. The total incidence of ras mutations was found to be 39% of the samples analyzed. Five patients showed evidence of more than one mutation. We obtained 22 marrow samples from patients at the time of disease progression or relapse, for whom a ras mutation was identified at diagnosis. In all cases, the ras mutation of the disease progression sample was identical to that found at diagnosis. In contrast, three of 25 patients who did not show any ras mutation at diagnosis acquired a ras mutation at the time of disease progression. No significant association was observed between any ras mutation and stage of disease, \( \beta \)-microglobulin levels, labeling index, or protein type. The mean tumor burden and median survival for patients with mutations of N-ras was indistinguishable from patients with no ras mutations. However, patients with K-ras mutations had a significantly higher mean bone marrow tumor burden at diagnosis than patients with no ras mutations (57% vs. 36%, \( P < 0.02 \)); and the median survival of patients with a K-ras mutation was significantly shorter (2.0 vs. 3.7 years, \( P < 0.02 \)). To determine if the status of ras mutations could affect treatment response, we examined patient survival on the three treatment arms of E9486. Although the presence of a ras mutation in the multidrug treatment, VBMCP alone, showed a marginal significance, neither the VBMCP, nor the addition of interferon-\( \alpha \) showed statistically significant survival differences between mutant and wildtype ras status. Interestingly, there appeared to be a statistically significant difference in survival of patients treated with VBMCP and alternating high doses of cyclophosphamide + prednisone. Patients with ras mutations had a median survival of 2.1 years; patients with wildtype ras had a median survival of 4.0 years (\( P < 0.01 \)).

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Multiple Myeloma (MM) is characterized by the clonal expansion of malignant plasma cells in the bone marrow. Although there are certain common clinical features such as anemia, bone lesions, hypercalcemia, and immunodeficiency,1 the disease shows significant heterogeneity with regard to morphology, disease progression, and response to therapy. This heterogeneity likely is due, in part, to differences in genetic abnormalities within the malignant clone, including various combinations of mutations in oncogenes and tumor suppressor genes.

The presence of activating mutations in the dominantly acting proto-oncogene ras, as well as inactivating mutations of tumor suppressor genes such as p53 and Rb have been reported in MM by a number of investigators.2-17 Somatic mutations involving the family of ras oncogenes are the most frequent. Activating mutations of ras at codons 12, 13, or 61 occur in several human cancers, including other hematologic malignancies such as acute myeloid leukemia, acute lymphoblastic leukemia, and blast crisis chronic myelogenous leukemia.12-16 Although the pathogenesis of MM is largely unknown, it has been suggested that ras mutations are associated with advanced stage disease.2-17

Previous studies of ras mutations in MM have shown discrepancies in ras mutation frequencies.13,17,18 and have had several limitations that prompted us to examine the issue in a large cooperative group trial. These limitations include few sample numbers from single institution studies, inclusion of both newly diagnosed patients and treated patients, lack of longitudinal studies of disease progression and survival, and potential false negative analysis of ras due to limits in assay sensitivities. In 1987, the Eastern Cooperative Oncology Group (ECOG) initiated a national protocol (E9486), with three randomized treatments of MM patients who had received no prior therapy. A total of 653 patients were enrolled on the clinical study and a total of 541 patient samples were submitted for ancillary laboratory analyses, including phenotypic and molecular characterizations of the marrow and blood. This provided a unique opportunity to establish clinical correlates such as staging, tumor burden, survival, and response to therapy, as well as relationships with laboratory parameters that serve as independent prognostic markers.

We have also recently developed an in vitro culture system in which different ras mutations have been introduced into a myeloma cell line.19,20 Our goal in these studies was to examine the ability of ras mutations to alter the growth properties of myeloma cells. We have shown that there appear to be distinctive properties imparted by N-ras61 and K-ras12 mutations on interleukin-6 (IL-6) and stromal cell-mediated growth and apoptosis.18 These results have

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prompted us to separately consider different ras mutations in the clinical cooperative group study.

We studied 346 untreated patients enrolled on E9486 for ras mutations at patient entry to the study. The course of disease was monitored with a median follow-up of 4.5 years for the 203 of the 653 patients still alive on the E9486 protocol. In this report we present an analysis of ras mutation frequency and clinical relationships. A number of the same patients were examined at disease progression or relapse to determine if there was any evidence for changes in ras status. Similar to results predicted from our in vitro myeloma cell culture system, we find significant differences in pretreatment tumor burden and survival between myeloma patients with mutations in N-or K-ras. Our study also showed an interesting trend that suggests possible differences in response to therapeutic regimens.

**MATERIALS AND METHODS**

**Patient selection and sample preparation.** Patients in this study were entered onto the Eastern Cooperative Oncology clinical phase III trial E9486 and companion laboratory study E9487, beginning in December 1987 through May 1992. The clinical and laboratory protocols were reviewed by the National Cancer Institute and were approved by the investigation review committees of the participating institutions. In this trial, 653 patients were enrolled and randomly assigned to receive regimen A: vincristine, carmustine (BCNU), melphalan, cyclophosphamide, and prednisone (VBMCP); regimen B: VBMCP plus alternating high-dose cyclophosphamide (HiCy); or regimen C: VBMCP plus α-IFN. The timeline for the protocol was reviewed by the Mayo Clinic Myeloma Tumor Biology Laboratory (directed by P.G.), the Mayo Clinic Myeloma Molecular Laboratory (directed by B.V.N.), the Myeloma Molecular Laboratory at the University of Minnesota (directed by B.V.N.), and the Molecular Laboratory sample were examined for ras mutation. Based on DNA availability and the sensitivity requirements, 346 untreated patient samples were selected for analysis of ras mutations. We first tested for N-ras61 mutations. Those samples negative for Nras61 mutation were then tested for N-ras12/13 (N = 129) and K-ras12/13 (N = 139) mutations. In assessing overall ras activating mutation frequencies, a mutation in any of the five codons was considered positive for an activating mutation, whereas patients were considered negative for ras mutations only if all five codons were wildtype.

**Oligonucleotide primers and polymerase chain reactions (PCR).** All the oligonucleotide primers used in this study were synthesized on the Milligen Bioresearch Model 8750 DNA synthesizer (Novato, CA). The sequences of the oligonucleotide primers used to amplify ras sequences were as previously described.22-25

Genomic DNA from patients’ bone marrow was amplified in a 100-μL reaction with 20 pmol of each primer, 2.5 U Taq polymerase (Amplitaq; Perkin Elmer-Cetus, Norwalk, CT), 200 μmol/L dNTPs (Pharmacia LKB Biotechnology, Uppsala, Sweden), 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), and 1.5 mmol/L MgCl2. The reactions were denatured at 95°C for 3 minutes and subjected to 30 cycles of amplification with denaturation at 94°C for 1 minute, annealing at 55°C to 60°C for 30 seconds, and extension at 72°C for 1 minute followed by final extension at 72°C for 15 minutes using a DNA Thermocycler (Perkin-Elmer/Cetus Corp, Emeryville, CA).

**Single-strand conformation polymorphism (SSCP) analysis.** Genomic DNA templates were amplified with primers22-26 end-labeled with [35S] γ-adenosine triphosphate (ATP) (4,500 mCi/mmol, ICN, CA) using T4 polynucleotide kinase (NEB, Beverly, MA). The PCR product was mixed with an equal volume of stop buffer (95% formamide, 20 mmol/L Tris-HCl, 0.05% bromophenol blue and 0.05% xylene cyanol), then denatured at 95°C for 5 minutes and chilled immediately on ice. A 4-μL aliquot of sample was loaded onto an 8% polyacrylamide/1X Tris borate EDTA (TBE) gel. Gels were run at 12 watts for 6 hours at 4°C or until the bromophenol blue dye had reached the bottom of the gel. Gels were dried on the gel dryer (HSGI, San Francisco, CA) and exposed to x-ray films at −70°C for 1 to 3 days.

**Dot blot hybridization.** The method was performed as described previously.27 Briefly, genomic DNA was amplified with primers flanking target sites and an 80-μL aliquot of the PCR product was then mixed with 320 μL of denaturing reagent (0.4 mol/L NaOH, 25 mmol/L EDTA). The mixture was heated at 95°C for 10 minutes and chilled on ice. The solution was then neutralized by the addition of 400 μL of 2 mol/L Tris (pH 7.5). A 100-μL aliquot of the
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Fig 2. Mutational analysis of ras mutations. Representative results are shown for each method described in the text. (A) SSCP analysis. Lane 1, negative control; lane 2, positive control (HL-60 cell line); lanes 3 to 14 myeloma patient bone marrow samples. (B) PCR cycle sequencing of a product that showed altered mobility in SSCP analysis. (C) Oligonucleotide specific hybridization. PCR amplified DNA was spotted onto nitrocellulose and hybridized with each mutant specific probe. Sequence difference at codon 61 for each probe is indicated on the left. The top row (CAA sequence) is the wildtype sequence, seen in all samples. (D) Restriction fragment polymorphism analysis of K-ras 12 mutation. The K-ras codon 12 containing PCR product was subjected to Bst NI digest. Samples with K-ras 12 mutation are resistant to the digest. Lane 0, no template control; lane +, positive control (SW480); lane -, negative control (HCT116); lanes 1 to 7, myeloma patient bone marrow samples.

denatured DNA was applied to a Zetabind nylon membrane (CUNO, Meriden, CT) using an S&S dot blot manifold (Schleicher & Schuell, Keene, NH) and hybridized to a panel of $^{32}$P y-ATP end-labeled oligonucleotide probes as described previously.23

Restriction fragment length polymorphism (RFLP). RFLP analysis of PCR amplification products was used to detect mutations at codons 12 and 13 of the K-ras oncogene as described previously.12 Briefly, genomic DNA was amplified with $^{32}$P y-ATP end-labeled primers and subjected to restriction enzyme digestion (BstNI for K-ras codon 12 mutation and Hph I for K-ras codon 13). The digestion products were separated by electrophoresis on an 8% polyacrylamide/1 TBE gel. The gels were then dried and exposed to x-ray films for 1 to 3 days.

Direct DNA sequencing. PCR products were sequenced with the cycle sequencing kit according to the manufacturers specifications (Epicentre Technologies, Madison, WI).

Statistical methods. Comparisons of quantitative patient characteristics such as tumor burden were performed using the Wilcoxon rank-sum test. Catagorical variables such as response to treatment were tested using Fisher's Exact Test. Survival curves were calculated using the method of Kaplan-Meier and compared by the log-rank test.

RESULTS

Incidence of ras mutations. Because the PCR methods we used amplified both normal and mutated alleles, we found by dilutional analysis of samples with known mutations that the sensitivity of the methods required greater than 2% clonality (data not shown). Genomic DNA from 346 MM bone marrow samples were selected based on availability of patient samples and the requirement for greater than 5% tumor involvement to ensure the PCR detection of ras mutations. When we initiated these studies, several previous reports had indicated N-ras codon 61 mutations were the most common
mutation in MM.\textsuperscript{2,8,17,18} For that reason, we began our studies by mutational analysis of N-ras\textsubscript{61} using the SSCP assay (see Fig 2A). Electrophoretic mobility that differed from wildtype controls indicated a mutation. Bands of altered mobility were excised from the gel and sequenced to confirm the site of mutation (Fig 2B). Alternatively, mutations were confirmed by spotting the amplified products on nylon membranes followed by oligonucleotide-specific hybridization (Fig 2C). At the time we initiated the study, we assumed that a mutation in N-ras\textsubscript{61} would be activating and if a mutation was detected, we did not analyze all of these samples at additional codons or ras genes. Moreover, we found that multiple mutations of ras were rare in over 150 subsequent analyses (see below). Samples that did not contain an N-ras\textsubscript{61} mutation were analyzed further for mutations of N-ras codons 12 and 13, and K-ras codons 12 and 13 by RFLP analysis (see Fig 2D) and direct sequencing. Previous reports indicated no, or only rare, incidence of K-ras\textsubscript{61} mutations,\textsuperscript{2,4,17} so this analysis was not included in our study.

The frequency of ras mutations we analyzed is summarized in Table 1. Although the number of analyses (N) for each codon is not identical, the sum of the individual codon frequencies suggests an incidence of ras of approximately 39%. This is higher than most previous reports,\textsuperscript{2,8,17,18} but the distribution is similar to other studies. N-ras codon 61 was the most frequent mutation, followed by mutation at codons 12 and 13 of K-ras. Five patients showed evidence for more than one ras mutation (two examples shown in Fig 2C, lanes 3 and 7). In one patient (lane 3, Fig 2C), the two distinct N-ras\textsubscript{61} mutations were of unequal intensities (by SSCP analysis) in the initial bone marrow sample. Interestingly, this patient went on to develop a plasmacytoma that contained only one of the two N-ras\textsubscript{61} mutations, suggesting there were likely distinct populations in our initial marrow sample (data not shown).

For 82 patients, we found no evidence for mutation in any of the five codons tested. This was not likely due to insufficient tumor content, as each sample was confirmed to contain greater than 5% tumor involvement, and the sensitivity of the method is 2%. Moreover, there was no failure to amplify or detect wildtype ras sequences.

To determine if there was any evidence for loss or changes in ras mutation at time of disease progression or relapse, we obtained 22 bone marrow samples that met ECOG criteria for progression or relapse, from patients for whom a ras mutation was identified at diagnosis. In all cases, the ras mutation of the disease progression sample was identical to that found in the diagnostic sample.

### Table 1. Incidence of ras Mutations in MM Patients

<table>
<thead>
<tr>
<th>Gene/codon</th>
<th>Analyzed at Entry to E9486</th>
<th>N</th>
<th>Mutations</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-ras\textsubscript{61}</td>
<td>346</td>
<td>50</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>N-ras\textsubscript{12}</td>
<td>129</td>
<td>6</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>N-ras\textsubscript{13}</td>
<td>129</td>
<td>5</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>K-ras\textsubscript{12}</td>
<td>139</td>
<td>16</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>K-ras\textsubscript{13}</td>
<td>139</td>
<td>7</td>
<td>5.0</td>
<td></td>
</tr>
</tbody>
</table>

Samples from 25 of the 82 patients who originally did not show any evidence for ras mutation in the initial diagnostic sample were reanalyzed at time of progression or relapse. In four cases, we found evidence for mutation of ras (2 N-ras\textsubscript{61}, 1 K-ras\textsubscript{12}, and 1 N-ras\textsubscript{12}) that was not found in the initial diagnosis sample. A representative pair of initial and relapse analysis is included in Fig 2C (lanes 4 and 5). The failure to detect ras mutations in the initial sample is unlikely, because in each case the tumor burden was greater than 10% (data not shown).

### Clinical features of MM patients with ras mutations

Analysis of possible relationships of ras status and a number of critical clinical parameters is presented in Table 2. In this analysis, patients with any ras mutation were compared with patients in which all codons tested were negative (N = 160). No significant association was observed between ras mutation status and stage of disease, β2 microglobulin levels, labeling index, or immunoglobulin protein type. The mean tumor burden and median survival for patients with mutant N-ras versus no mutation in N-ras was indistinguishable (Table 3). However, patients with a K-ras mutation had significantly higher mean tumor burden at diagnosis than patients with no ras mutations or N-ras mutations (57% vs 36% and 39%, respectively; P < .02).

The survival curves from which the data in Table 3 were calculated are shown in Figs 3 and 4. As shown in Fig 3A, the survival curves comparing N-ras mutation versus wildtype N-ras are completely overlapping, with median survival calculated as 3.7 years for both groups of patients. In contrast, the survival curve for patients with K-ras mutation show a significant difference (Fig 3B), with a median sur-
vival of 2.0 years, compared with 3.7 years in patients with no \( \text{K-ras} \) mutations (\( P < .02 \)).

**Analysis of \( \text{ras} \) status and treatment protocol.** To evaluate whether myelomas with \( \text{ras} \) mutations may show differences in response to the different E9486 therapies, we examined survival of patients in each of the three treatment regimens in E9486. As described in the Materials and Methods section and shown in Fig 1, the three treatments included (A) VBMCP, (B) VBMCP with alternating high-dose cyclophosphamide + prednisone, or (C) VBMCP + \( \alpha \)-interferon. Because the numbers of patients are too low to evaluate \( \text{ras} \) subgroups for each therapy, we examined survival simply on the basis of wildtype versus any mutation in \( \text{K-ras} \) or \( \text{N-ras} \) (Fig 4). Neither the VBMCP alone, nor the addition of \( \alpha \)-interferon showed statistically significant survival differences between mutant and wildtype \( \text{ras} \) status (Fig 4A and C). Interestingly, there appeared to be a statistically significant difference in comparing \( \text{ras} \) status and survival of patients treated with VBMCP and alternating high-dose HiCy (Fig 3B). The median survival for patients with \( \text{ras} \) mutations in this treatment group was 2.1 years compared with 4.0 years for patients with no evidence of \( \text{ras} \) mutations (\( P < .01 \)). This difference appeared to be the result of a surprising early, extended survival among patients with no \( \text{ras} \) mutation, as well as a precipitous drop in survival around 2 years among patients with a \( \text{ras} \) mutation. It is important to point out that, although this comparison reached statistical significance, due to the relatively low number of patients, this can only be viewed as an interesting trend at this time.

**DISCUSSION**

In this report, we provide the first analysis of \( \text{ras} \) mutations associated with MM, treated with chemotherapy in a large cooperative group trial. This study provides some distinct advantages including: uniform treatment and follow-up; relationships with other laboratory studies; relationship to diagnosis, response, relapse and survival; and rigorous review and analysis by the ECOG Laboratory Committee, data managers, study chair, and statisticians of diagnosis, relapse, and response.

Other studies have reported significant discrepancies in the frequency of \( \text{ras} \) mutations detected in MM patients, ranging from 9% to 32%.

In addition, no study has focused on initial diagnostic samples and longitudinal follow-up of patients to establish clinical relationships. Our analysis shows a somewhat higher frequency of \( \text{ras} \) mutations in MM than most studies, but is consistent with other reports in identifying \( \text{N-ras} \) and \( \text{K-ras} \) mutations as the most common sites of activating mutations. In our case, we only examined patients who demonstrated greater than 5% malignant cells involvement in the same sample we used for \( \text{ras} \) analysis, and this may create some bias. However, this significantly reduces the number of false negatives, which we felt was an important consideration. Finally, ECOG diagnostic criteria for myeloma requires that patients have symptomatic, active disease before protocol entry. In contrast to other reports, we found evidence for \( \text{ras} \) mutations in myeloma cells from patients at all stages of the disease, with no significant association with stage III disease.

\( \text{Ras} \) genes are frequently altered in premalignant breast and colon adenomas, leading to the concept that \( \text{ras} \) may act early in a multistep carcinogenesis. Although \( \text{ras} \) mutations have been observed in terminally differentiated plasma cells, it has been proposed that mutations may actually occur in

**Table 3. Relationships Between \( \text{ras} \) Status, Tumor Burden, and Survival for MM Patients on E9486**

<table>
<thead>
<tr>
<th>Mean tumor burden (%)</th>
<th>( \text{Wild Type} )</th>
<th>mut( \text{N-ras} )</th>
<th>mut( \text{K-ras} )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>36</td>
<td>39</td>
<td>57</td>
<td>.012</td>
</tr>
<tr>
<td>Median Survival (yrs)</td>
<td>3.7</td>
<td>3.7</td>
<td>2.0</td>
<td>.018</td>
</tr>
</tbody>
</table>

\( \text{mutK-} \) or \( \text{N-ras} \)

<table>
<thead>
<tr>
<th>Survival by treatment (yr)</th>
<th>( \text{A) VBMCP} )</th>
<th>( \text{B) VBMCP + HiCy} )</th>
<th>( \text{C) VBMCP + INF-\alpha} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{A) VBMCP} )</td>
<td>2.4</td>
<td>4.6</td>
<td>3.2</td>
</tr>
<tr>
<td>( \text{B) VBMCP + HiCy} )</td>
<td>( &gt;P = .06 )</td>
<td>2.1</td>
<td>2.5</td>
</tr>
<tr>
<td>( \text{C) VBMCP + INF-\alpha} )</td>
<td>( &gt;P = .04 )</td>
<td>.014</td>
<td>.492</td>
</tr>
</tbody>
</table>

Fig 3. (A) Survival comparison of patients who were determined to have wildtype (dotted line) or a mutated \( \text{N-ras} \) (solid line). (B) Survival comparison of patients who were determined to have wildtype (dotted line) or mutated \( \text{K-ras} \) (solid line).
myeloma precursor cells. However, because ras mutations are not found in all myelomas, it is unlikely that a ras mutation represents an early, obligate step in the pathogenesis of the disease. It has been suggested that monoclonal gammopathies of undetermined significance (MGUS) may represent a premalignant phase of MM. Assuming detection of ras mutations was not below levels of assay sensitivities, the apparent absence of ras mutations in MGUS recently reported suggests that ras does not likely play a role in the development of the premalignant lesion, as has been proposed for adenomas of the colon. Moreover, the fact that in a limited longitudinal study of 25 patients we found evidence for acquired ras mutations, suggests ras is not an initiating event in the disease, but may be associated with disease progression. It will be interesting to determine the frequency of ras mutations that may be acquired in those MGUS patients that ultimately progress to active myeloma.

Based on our analysis, it does not appear that mutations in N- or K-ras are associated with other prognostic markers of aggressive disease, such as beta-2 microglobulin or elevated labeling index. However, on morphologic review, it was found that patients with any ras mutation had a higher probability of having plasmablastic (PB) morphology (P < .05); and PB patients had a significantly shorter median survival (P < .0001) (ECOG Myeloma Laboratory Group, submitted).

We found no statistically significant relationship between N-ras status and tumor burden or survival. However, there was a significant association of tumor burden and survival with the codon 12/13 mutation status of the K-ras gene. This result is consistent with predictions we had made based on our analysis of N- or K-ras mutations in an in vitro myeloma cell line. By introducing different ras mutations into the myeloma cell line, ANBL6, we found that in the presence of IL-6, cells with a K-ras12 mutation proliferated significantly greater than those with wildtype or an N-ras 61 mutation. Both these in vitro results and the clinical relationship reported here suggest there are distinctive properties associated with the activation of K-ras versus activation of N-ras.

One of the interesting results we observed in this study was the association of ras status with survival in one of the three treatment arms of the E9486 trial. It is not clear why the survival showed a statistically significant relationship with ras status for patients treated with VBMCP plus alternating high doses of HiCy, nor whether the difference would be substantiated with increased numbers of patients. This treatment was the only one of the three that resulted in replacing VBMCP at cycles 3 and 5 with single agent doses (see Fig 1). It is possible that maintaining the multidrug regimens of the other two arms reduced the ras effect. Because HiCy induces significant neutropenia, many patients on this treatment also received granulocyte colony-stimulating factor (G-CSF), a potent hematologic growth factor. IL-6 serves as a potent growth factor for myeloma cells and in our in vitro myeloma system, we consistently noted that mutations in ras significantly augmented the IL-6 effect, as evidenced by increased [3H]-thymidine uptake and proliferation, as well as decreased apoptosis. In a recent study, it was reported that myeloma cell lines show augmentation of IL-6-induced proliferation by addition of GM-CSF. G-CSF and granulocyte macrophage (GM)-CSF-induced proliferation was also linked to Ras signal transduction. Based on the results from another recent study, it has been suggested that G-CSF may increase tumor mobilization to
the periphery. This has prompted us to consider the possibility that K-ras mutation effects may be augmented by growth factors such as G-CSF. This possibility is now being tested in the myeloma culture system we have developed.

Other known oncogenes and tumor suppressor genes have been found to have altered expression or mutation effects in myeloma plasma cells. Thus, it is likely that the analysis of a single oncogene provides only a limited view of the various genetic alterations that impact on myeloma proliferation, disease progression, and response to therapy.

Our study of ras mutations in the clinical trial was prompted, in part, by the results of an in vitro myeloma system designed to determine effects of activating mutations on growth properties and apoptosis. The results presented in this report suggest that the development of an in vitro system can direct subsequent analysis of the clinical outcomes. Similarly, the results of the clinical analysis has also led to hypotheses that can now be directly tested in cell culture systems.

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