Farnesyltransferase inhibitor tipifarnib is well tolerated, induces stabilization of disease, and inhibits farnesylation and oncogenic/tumor survival pathways in patients with advanced multiple myeloma


Patients with multiple myeloma (MM) with mutated RAS are less likely to respond to chemotherapy and have a shortened survival. Therefore, targeting RAS farnesylation may be a novel approach to treatment of MM. We evaluated the activity and tolerability of the farnesyltransferase (FTase) inhibitor tipifarnib (Zarnestra) in a phase 2 trial as well as its ability to inhibit protein farnesylation and oncogenic pathways in patients with relapsed MM. Forty-three patients (median age, 62 years [range, 33-82 years]) with a median of 4 (range, 1-6) chemotherapy regimens entered the study. Tipifarnib, 300 mg orally twice daily, was administered for 3 weeks every 4 weeks. The most common toxicity was fatigue occurring in 66% of patients. Other toxicities included diarrhea, nausea, neuropathy, anemia, and thrombocytopenia. Sixty-four percent of the patients had disease stabilization. Treatment with tipifarnib suppressed FTase (but not geranylgeranyltransferase 1) in bone marrow and peripheral blood mononuclear cells and also inhibited the farnesylation of HDJ-2 in unfractionated mononuclear cells and purified myeloma cells. Inhibition of farnesylation did not correlate with disease stabilization. Finally, tipifarnib decreased the levels of phosphorylated Akt and STAT3 (signal transducer and activator of transcription 3) but not Erk1/2 (extracellular signal regulated kinase 1 and 2) in bone marrow cells. We conclude that tipifarnib is tolerable, can induce disease stabilization, and can inhibit farnesylation and oncogenic/tumor survival pathways.

Introduction

Multiple myeloma (MM) is a hematologic malignancy characterized by the preferential accumulation of plasma cells in the bone marrow. It is the second most common hematologic malignancy in adults, with 14,600 new cases diagnosed in the United States yearly. In recent years it has become evident that the interactions between the myeloma cells and the stromal cells in the tumor microenvironment play an important role in tumor growth, drug resistance, and bone disease. This understanding has lead to the development of novel treatments that are not only able to induce myeloma cell cytotoxicity but also to interfere with myeloma-stromal cell interactions either directly or by interrupting signal transduction pathways involved in disease activity and progression. An important mediator of myeloma-stromal cell interaction is interleukin-6. Myeloma cell adhesion to stromal cells induces interleukin-6 secretion, which in turn mediates myeloma cell growth through the activation of signal transduction pathways, such as the JAK/STAT (Janus kinase/signal transducer and activator of transcription), PI3K (phosphatidylinositol 3-kinase)/Akt, and Ras/Raf/Mek/ERK1-2 (extracellular signal regulated kinase 1 and 2). These signal transduction pathways that are downstream from IL-6 represent potential treatment targets in multiple myeloma. Among these pathways, the Ras oncogene is of particular interest because of its frequent mutation in MM. Neri et al reported an incidence of Ras mutations in myeloma to be 30% at diagnosis and increasing to 50% at time of disease progression. These mutations most commonly affect the N-Ras gene, although less common K-ras mutations are associated with particularly poor survival.

Ras and Rho families belong to a large number of low molecular weight GDP/GTP (guanosine diphosphate/guanosine triphosphate) binding GTPases (guanosine triphosphatases) that require a lipid posttranslational modification for their cellular localization and biologic activities. For example, Ras proteins (H-, N-, and K-Ras) are modified at their carboxyl termini with the 15-carbon lipid farnesyl, whereas RhoA and RhoC are modified with the 20-carbon geranylgeranyl. These GTPases are intimately involved in growth factor signal transduction, modulation of proliferation and differentiation, and the
balance between survival and programmed cell death. In addition to their pivotal role in normal physiologic processes, these proteins are also intricately involved in oncogenesis, invasion, and metastasis. The ability of Ras and Rho proteins to cause malignant transformation depends on their farnesylation or geranylgeranylation. These lipid posttranslational modifications are catalyzed by farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I), which attach covalently farnesyl and geranylgeranyl, respectively, to cysteine side chain of polypeptide that end with the tetrapeptide consensus sequence CAAX (C indicates cysteine; A, aliphatic amino acid residues; and X, any residue). FTase prefers methionine or serine, and GGTase I prefers leucine at the X position of CAAX. However, under certain circumstances, this substrate preference is not strictly respected. For example, although H-, N-, and K-Ras proteins are normally farnesylated, K-Ras and possibly N-Ras become geranylgeranylated when FTase is blocked in human cancer cells. Furthermore, RhoB, which has CKVL as a CAAX box, is naturally farnesylated and geranylgeranylated.

The fact that Ras and Rho proteins require prenylation for malignant transformation prompted us and others to make FTase and GGTase I inhibitors (FTIs and GGTIs). Both FTIs and GGTIs are effective suppressors of human cancer growth, but their mechanisms of action differ. GGTIs arrest cells at the G1 phase of the cell cycle through a mechanism involving induction of p21\(^{WAF1}\), inhibition of cyclin-dependent kinases CDK2 and CDK4, and pRb hypophosphorylation. In contrast, FTIs either accumulate cancer cells in prometaphase during mitosis, have no effect on cell cycle distribution, or, less frequently, induce G1 phase arrest. Recently, we have shown that FTIs inhibit growth and induce apoptosis in myeloma tumor cells that are resistant to conventional chemotherapy. In some human cancer cells FTIs can induce apoptosis by a mechanism involving blockade of the PI3K/Akt pathway. Furthermore, FTIs have been shown to have antitumor activity in many animal models, including nude mouse xenografts, oncogenic Ras transgenic mice, and carcinogen-induced lung oncogenesis models. Because of their outstanding antitumor efficacy and limited toxicity in animal models, FTIs have moved into clinical trials. Tipifarnib, a potent and selective FTI, is presently undergoing phase 2/3 trials. As a single agent in a phase 2 trial of patients with metastatic breast cancer, clinical activity was seen in 24% of patients. Furthermore, in a recent phase 1 study, 29% of patients with refractory or relapsed acute leukemia responded to tipifarnib. A similar response rate (30%) was also observed in patients with myelodysplastic syndrome treated with tipifarnib.

We now report the first clinical trial of tipifarnib in patients with advanced MM. This hypothesis-driven phase 2 clinical trial not only evaluated the activity and tolerability of tipifarnib but also attempted to correlate clinical activity to enzymatic activity, protein farnesylation, and oncogenic/tumor survival pathways. Our results show that tipifarnib can induce disease stabilization in patients with advanced multiple myeloma at doses that are well tolerated (600 mg daily, 3 weeks on/1 week off schedule), and that this dose is sufficient to inhibit the biochemical target FTase, protein farnesylation, and oncogenic/tumor survival pathways but not the closely related enzyme GGTase I.

**Patients, materials and methods**

**Patients and treatments**

Between January 2001 and June 2002, 43 consecutive, eligible patients with previously treated and progressive myeloma began treatment with oral tipifarnib as a single agent, after providing written informed consent. All patients entered in study had evidence of disease progression/relapse as documented by a rising M-protein or progression in bone disease. Therefore, all patients had relapsed myeloma that required a change in management. All patients were treated according to the phase 2 protocol approved by institutional review boards of the H. Lee Moffitt Cancer Center/University of South Florida and Mayo Clinic as well as the Cancer Treatment Evaluation Program of the National Cancer Institute. Patients were excluded if they had renal insufficiency, defined as a creatinine higher than 1.5 times the upper limit of normal, if the liver function tests were more than twice the upper limits of normal, or if they had none secretory myeloma. Comitant use of bisphosphonates and growth factors were allowed. FTI was supplied in 100-mg capsules and was administered at a dose of 300 mg twice/d for 3 weeks followed by 1 week of no treatment. Each treatment cycle had a duration of 4 weeks. The dose was to be escalated to 400 mg twice/d after 1 month of treatment if no grade 3 toxicity were encountered. Because the first 4 patients treated developed grade 3 fatigue on dose escalation, the protocol was amended to continue patients on the initial dose of 300 mg twice/d without dose escalation. Patients were continued on treatment indefinitely unless they developed disease progression or dose-limiting toxicities that required drug discontinuation. Data were analyzed as of November 2003 when the duration of treatment ranged from 1 to 26 months (median, 4 months).

**Patient evaluation**

The pretreatment evaluation included complete blood counts, renal and liver function tests, serum protein electrophoresis (SPEP), urine protein electrophoresis (UPEP), quantitative immunoglobulins, bone survey, and bone marrow aspiration and biopsy. Patients were evaluated for response after 2 months of treatment and continued on study if they had disease response or stabilization according to Southwest Oncology Group (SWOG) criteria. Follow-up studies included monthly blood counts, blood chemistries, and quantitation of monoclonal protein.

**Assessment of response**

The primary end point of the study was to determine the rate of objective response and disease stabilization. Responses were defined according to modified SWOG criteria. Disease progression was defined as a 25% increase in the serum M-component confirmed by a second measurement obtained within 1 to 4 weeks of the first measurement, or an increase in the 24-hour urine M-component by more than 50%, confirmed by a second measurement. Other criteria for disease progression included the need to administer radiotherapy, new lytic bone lesions, enlargement of existing bone lesions, or new soft tissue plasmacytomas.

**Assessment of adverse effects**

All patients were included in the evaluation of adverse effects. Patients received diaries to record adverse events. Patients were evaluated on a monthly basis by investigators, and adverse events were recorded according to Common Toxicity Criteria Version 2 (CTC.2) criteria.

**Laboratory correlatives studies**

**Preparation of PBMN cells.** Peripheral blood mononuclear (PBNN) cells were prepared from whole blood in Vacutainer CPT tubes as follows: After mixing the blood in the anticoagulant-containing CPT tube, the samples were spun at 1600g for 30 minutes. The upper half of the plasma layer was discarded, and the PBNN layer was collected. PBNN cells were then washed with sterile phosphate-buffered saline (PBS), and the pellets were frozen for subsequent use.

**Preparation of bone marrow cells.** Bone marrow mononuclear cells were prepared from aspirates by centrifuging at 600 g for 5 minutes, resuspending the cell pellet with PBS, and overlaying on an equal volume of Ficoll. After centrifuging at 800 g for 20 minutes, the interface was collected, resuspending in PBS, and centrifuged at 1500 rpm for 5 minutes. After an additional wash with PBS, the cell pellets were frozen at −80°C for future use. Alternatively, bone marrow mononuclear cells were enriched for CD138+ myeloma cells by immunomagnetic bead separation. After
CD138-selected cells were washed in serum-free medium, a small aliquot was saved for analysis of CD138 expression by flow cytometry; the remaining was lysed in 6 M guanidine hydrochloride under reducing conditions as previously described.22

Effects of tipifarnib on FTase and GGTase I enzymatic activities

Patient bone marrow cells and PBMN cells were obtained at baseline and 3 weeks after treatment with FTI. Cells were sonicated in 250 to 500 μL sonication buffer (50 mM Tris (tris(hydroxymethyl)aminomethane)-HCl [pH 7.5], 1 mM EDTA (ethylenediaminetetraacetic acid), 1 mM EGTA (ethylene glycol tetraacetic acid), 1 mM DTT (dithiothreitol), 2 mM PMSF (phenylmethylsulfonyl fluoride), 10 μg/mL aprotinin, 10 μg/mL soybean trypsin inhibitor, and 25 μg/mL leupeptin). When enough material was available, a portion of a sample was prepared for Western blotting by adding an equal volume of 2 × lysis buffer (60 mM HEPES (N-2-hydroxyethylpiperazine-Ν′-2-ethanesulfonic acid)-OH [pH 7.5], 20 mM NaCl, 10 mM MgCl₂, 50 mM NaF, 2 mM EGTA, 2% Triton X-100, and 20% glycerol), incubating on ice for 30 minutes, centrifuging at 12 000 g for 15 minutes, and preparing the supernatants for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting as described in “Effects of tipifarnib treatment on protein prenylation and signal transduction pathways by Western blotting.” The other portion of the sonicate was used for FTase and GGTase I enzymatic assays as follows: The sonicates were spun at 12 000 g for 30 minutes. The resulting supernatants were spun at 60 000 g for 1 hour, and the postmicrosomal supernatants were collected. The ability of FTase and GGTase I to transfer [3H]farnesyl and [3H]geranylgeranyl from [3H]farnesylpyrophosphate (FPP) and [3H]geranylgeranylpip DISPERSIONS (GGPP) to recombinant H-Ras-CVLS and H-Ras-CVIL, respectively, was determined as described by us previously.20,21 Briefly, aliquots of postmicrosomal supernatants containing 20 μg protein were incubated for 30 minutes at 37°C in 50 mM Tris- HCl (pH 7.5), 50 μM ZnCl₂, 20 mM KCl, 1 mM DTT, and 3 mM MgCl₂ in the presence of either 0.5 μCi (0.0185 MBq) [3H]FPP and 0.5 μg/mL H-Ras-CVLS (FTase) or 0.5 μCi (0.0185 MBq) [3H]GGPP and 0.25 μg/mL H-Ras-CVLL (GGTase I). After the reaction was stopped with 10% SDS, the proteins were precipitated with 30% trichloroacetic acid (TCA) and collected on glass microfilter filters as described previously.21

Effects of tipifarnib treatment on protein prenylation and signal transduction pathways by Western blotting

Lysates prepared from bone marrow or peripheral blood mononuclear cells or from CD138-selected myeloma cells were separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with various antibodies or from CD138-selected myeloma cells were separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with various antibod- to CD138 expression by

Results

Patient characteristics

Table 1 shows a summary of the characteristics of the patients and disease history for all 43 patients enrolled in study. The age of patients entered in study ranged from 33 to 82 years, with a median of 62 years. The male-female ratio of patients was 1.4:1. Patients had a received a median of 4 (range, 1-6) treatment regimens prior to enrollment. Fifty-two percent of patients were refractory to prior therapy; and more than half had received thalidomide as well as high-dose chemotherapy and transplantation prior to enrollment in the study. At the time of data analysis the median duration of treatment for all enrolled patients was 4 months.

Efficacy

Of the 43 patients that enrolled in this study, 7 were not evaluable, because they did not complete at least 2 cycles of treatment for reasons other than disease progression (toxicity [n = 1], consent withdrawal [n = 4], and failure to complete 1 cycle of treatment [n = 2]). Efficacy analysis included the 36 evaluable patients that have received 2 or more cycles of FTI and is reported after 2 cycles of treatment. As shown in Table 2, no patient showed a complete or partial response. Thirty-six percent of patients had disease progression. However, 64% of patients showed stabilization of disease, which was defined as a less than 50% reduction in M-component (4 patients with 25%-49% reduction, 13 patients with 1%-25%, and 6 patients with no change in M-protein levels). In the 4 patients with M-protein level decrease of 25% to 49%, the reduction persisted for 26, 14, 7, and 3 cycles of treatment. The median time to progression from the start of treatment from those patients who achieved disease stabilization is 4 months and ranged from 2 to 26 months. Forty percent of patients with stable disease remained on treatment and stable for at least 5 months. The number of treatment cycles received per patient was 1 to 26 (median, 4). In one patient treatment was started for a relapse occurring after high-dose chemotherapy with stem cell support. The patient had a 3- to 4-cm round plasmacytoma in the right forehead region at the initiation of the medication. The tumoral collection of cells could be easily palpable and arose from a growth in the skull plate. On treatment initiation the tumoral mass was no longer palpable, and a large lytic lesion was all that was left in the original location of the plasmacytoma. The area went from being a convex palpable soft mass to a concave large defect in the skull. The patient has been

Table 1. Patients’ characteristics, N = 43

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. treated</td>
<td>43</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>62 (33-82)</td>
</tr>
<tr>
<td>Sex ratio, M/F</td>
<td>1.4:1</td>
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<tr>
<td>Median prior treatment (range)</td>
<td>4 (1-6)</td>
</tr>
<tr>
<td>Refractoriness to prior treatment, %</td>
<td>52</td>
</tr>
<tr>
<td>Prior thalidomide, %</td>
<td>54</td>
</tr>
<tr>
<td>Prior BMT, %</td>
<td>54</td>
</tr>
<tr>
<td>Median cycles completed/patient (range)</td>
<td>4 (1-26)</td>
</tr>
</tbody>
</table>

BMT indicates bone marrow transplantation.
Table 3. Drug-related adverse events

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Overall, %</th>
<th>Grade 3-4, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>66</td>
<td>12.5</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td>Anemia</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Neuproside</td>
<td>29</td>
<td>8</td>
</tr>
<tr>
<td>Nausea</td>
<td>25</td>
<td>4</td>
</tr>
</tbody>
</table>

*CTC 2.0 criteria.

receiving the medication for 16 months without evidence of further progression (data not shown).

Safety

Analyses of safety were based on data from all 43 patients enrolled in study. Table 3 shows treatment-related adverse events. The most frequently reported side effect was fatigue in 66% of patients. Fatigue was grade 1-2 in all patients after the protocol was amended to keep the dose of the drug at 300 mg twice/d throughout. Grade 3 fatigue was encountered only in the patients in whom the dose of FTI was escalated to 400 mg twice/d. The next most commonly encountered toxicity was diarrhea, seen in 46% of patients. Other toxicities included anemia, thrombocytopenia, nausea, and neuropathy. These toxicities were all low-grade events and, therefore, not dose limiting.

Tipifarnib treatment inhibits Ftase, but not GGTase I, enzymatic activity in bone marrow cells from patients with multiple myeloma

To determine whether tipifarnib reached and inhibited its intended biochemical target, Ftase enzyme activity was determined in bone marrow cells that were collected from patients prior to the first tipifarnib dose (baseline or B) and during the third week after initiation of tipifarnib treatment (T). Bone marrow cells were isolated and processed for Ftase and GGTase I enzyme activity assays as described in “Materials and methods.” Figure 1A shows that tipifarnib treatment resulted in potent (70%-90%) inhibition of Ftase enzymatic activity. Figure 1A also shows that there is no correlation between the ability of tipifarnib to inhibit Ftase in unfraccionated marrow mononuclear cells and clinical activity. For example, Ftase activity was inhibited potently (more than 80%) in patients that progressed (PD) (ie, patient 2) as well as those that had stable disease (SD) (ie, patient 7). To determine whether tipifarnib treatment of patients with advanced multiple myeloma resulted in selective inhibition of Ftase over the closely related enzyme GGTase I, we also analyzed GGTase I enzymatic activity in bone marrow cells. Figure 1B shows GGTase I enzymatic activity levels at baseline before initiation of treatment and during the 3rd week of tipifarnib treatment. In contrast, to Ftase activity, GGTase I activity was not inhibited following tipifarnib treatment. In fact, there was a modest increase in GGTase I activity in samples from 6 of 8 patients (Figure 1B). However, the modest increase in GGTase I activity did not correlate with clinical activity.

Tipifarnib treatment inhibits Ftase enzymatic activity and HDJ-2 protein farnesylation in PBMM cells from patients with multiple myeloma

Figure 1 clearly demonstrates that tipifarnib treatment is effective at inhibiting Ftase enzyme activity in bone marrow cells. To determine whether tipifarnib treatment also resulted in inhibition of Ftase enzyme activity in peripheral blood mononuclear cells, blood was collected prior to, and during the 3rd week of tipifarnib treatment, and PBMM cells were prepared and processed for Ftase enzyme activity assays as well as HDJ-2 protein farnesylation as described in “Materials and methods.” Figure 2A shows the levels of Ftase activity at baseline (B) and during treatment (T) in PBMM cells from 3 patients. Treatment with tipifarnib resulted in inhibition of Ftase in all 3 patients. To determine whether inhibition of Ftase activity resulted in inhibition of protein farnesylation in PBMM cells, we evaluated the effects of tipifarnib treatment on HDJ-2 farnesylation. HDJ-2 is exclusively farnesylated and easily detectable, and inhibition of its farnesylation results in band shift in SDS-PAGE
Western immunoblotting. Hence, HDJ-2 farnesylation is a good surrogate biochemical marker for FTase activity. Figure 2B shows that prior to tipifarnib treatment at baseline (B), HDJ-2 protein from PBMN cells migrated as a single band. In contrast, in patients treated with tipifarnib, HDJ-2 migrated as a double band, one which migrated slower (unprocessed [U] non-farnesylated) and the other faster (processed [P] farnesylated) (Figure 2B). Therefore, in all 3 patients analyzed tipifarnib treatment resulted in inhibition of FTase (Figure 2A) and HDJ-2 farnesylation (Figure 2B).

Tipifarnib inhibits HDJ-2 protein farnesylation and oncogenic and tumor survival pathways in bone marrow of patients with multiple myeloma

To determine whether inhibition of protein farnesylation may result in disruption of signaling pathways in patients with advanced multiple myeloma, bone marrow cells were processed for Western immunoblotting as described in “Materials and methods.” We determined the effects of tipifarnib on the farnesylation of HDJ-2 as well as the geranygeranylation of Rap1 to determine selectivity. In addition, the effects of tipifarnib treatment on P38/Akt, JAK/STAT3, and Mek/Erk1/2 signal transduction pathways was also determined by measuring the levels of phosphorylated and total levels of Akt, STAT3, and Erk1/2. Figure 3 shows that, although in every patient tested treatment with tipifarnib resulted in inhibition of HDJ-2 farnesylation, Rap1 geranygeranylation was not affected, demonstrating that treatment with tipifarnib inhibited its intended target FTase selectively, without affecting the closely related enzyme GGTagase1 in bone marrow. Furthermore, tipifarnib treatment affected oncogenic and tumor survival pathways differentially. For example, in patient 7, tipifarnib partially inhibited the levels of phospho-Akt, phospho-STAT3, and phospho-Erk1/2 without affecting the levels of total Akt, STAT3, and Erk1/2. In patient 8, phospho-Akt levels were decreased with a concomitant increase in Akt levels, whereas both phospho-STAT3 and STAT3 levels decreased with little effects on both phospho-Erk1/2 and Erk1/2 levels (Figure 3). In contrast, in patients 1 and 3, tipifarnib treatment had no effect on phospho-Akt and total Akt levels but decreased phospho-STAT3 levels with little effect on phospho-Erk1/2 and Erk1/2 levels.

To determine whether tipifarnib treatment inhibits farnesylation in the malignant plasma cells, CD138-selected plasma cells were prepared from 3 patients prior to therapy and again on day 21. Flow cytometry confirmed that each of these samples contained more than 90% plasma cells. Because of limited cell numbers, only a small number of antigens could be examined by immunoblotting. HDJ-2 analysis demonstrated that in all 3 patients there was an increase in unprocessed HDJ-2 after tipifarnib treatment (Figure 4), confirming the inhibition of FTase activity in the malignant plasma cells.

Ras mutation status does not correlate with clinical activity

Ras mutation status was determined prior to treatment in 20 of the 43 patients. As shown in Table 4, 7 (35%) of the 20 patients examined had a Ras mutation identified. The frequency of N-Ras and K-Ras mutation was 25% and 10%, respectively. There was no correlation with response to treatment.

Discussion

This is the first report of a phase 2 clinical trial of a farnesyltransferase inhibitor in advanced MM. We found that tipifarnib at a dose of 300 mg twice/d induces stabilization of disease in a significant proportion of patients with advanced myeloma. The drug is well tolerated, with grade 1-2 fatigue being the most common toxicity encountered in this patient population. Although the median duration of disease stabilization was only 4 months, this population of patients was heavily pretreated, and 50% were refractory to prior treatment and had already received prior thalidomide and bone marrow transplant. Thirteen of 36 evaluable patients (36%) had disease progression and 23 of 36 (64%) had disease stabilization. The median time to progression from the start of treatment from those patients who achieved disease stabilization is 4 months and ranged from 2 to 26 months. Forty percent of patients with stable disease remained stable at least 5 months. Interestingly, one of the patients with a significant amyloid component with subcutaneous nodules, organomegaly, and peripheral edema had resolution of these signs after treatment, suggesting that the drug may have a role in the treatment of amyloidosis.

Table 4. Frequency of Ras mutations, N = 20

<table>
<thead>
<tr>
<th>Mutation</th>
<th>N (%)</th>
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<tbody>
<tr>
<td>K-Ras</td>
<td>2 (10)</td>
</tr>
<tr>
<td>N-Ras</td>
<td>5 (25)</td>
</tr>
<tr>
<td>No mutation</td>
<td>13 (65)</td>
</tr>
</tbody>
</table>

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Our studies show that treatment of patients with advanced MM with tipifarnib 300 mg twice daily resulted in inhibition of protein farnesylation in bone marrow mononuclear cells, PBMN cells, and purified plasma cells. The partial inhibition of HDJ-2 protein farnesylation with potent inhibition of FTase enzymatic activity was observed by others and has been suggested to be due to a high turnover rate of the HDJ-2 protein. Consistent with our results, Karp et al\textsuperscript{30} also showed in leukemias that tipifarnib treatment resulted in inhibition of FTase activity as well as inhibition of farnesylation of HDJ-2. We have also shown that the inhibition of FTase is selective in that treatment with tipifarnib did not result in inhibition of the closely related GGtase I. In fact in 6 of the 8 patients analyzed, GGtase I enzyme activity was modestly increased and did not change in the other 2 patients. Whether this finding is biologically significant is not known, but clearly the clinical activity of tipifarnib is not dependent on its modulation of GGtase I activity. Furthermore, the ability of tipifarnib to inhibit FTase does not translate into clinical activity. Indeed, from Figure 1A, one can see that FTase activity was potently inhibited in patients with disease stabilization (SD) as well as those with disease progression (PD). This suggested that farnesylated proteins are not required for tumor survival in some patients with multiple myeloma, whereas in those patients in which tipifarnib induced disease stabilization, protein farnesylation appears to be important. Data from a limited number of patients (4) of this phase 2 study show that treatment with tipifarnib affected differentially pivotal oncogenic and tumor survival pathways. These data show that tipifarnib inhibited phospho-STAT3 levels in all 4 patients, but this inhibition did not correlate with clinical activity, because patients 1 and 3 progressed (PD), whereas patients 7 and 8 stabilized (SD). Similarly, the effects on phospho-Erk1/2 levels did not correlate either. In contrast, tipifarnib treatment inhibited the levels of phospho-Akt levels in patients 7 and 8 that stabilized but not in patients 1 and 3 which progressed. Because of the limited number of samples that could be successfully analyzed, the significance of this observation cannot be determined at this time. Nevertheless, this observation is consistent with our preclinical data in cultured human cancer cells in which induction of apoptosis by FTIs required inhibition of the PI3K/Akt pathway.\textsuperscript{27} However, it is critical in subsequent trials that more patients be analyzed and that enough multiple myeloma cells be isolated from bone marrow to determine the effects of tipifarnib on the above-mentioned signaling pathways in malignant cells.

We conclude that tipifarnib is well tolerated and causes disease stabilization in some patients with advanced multiple myeloma, and that 300 mg twice daily is sufficient to inhibit FTase activity, protein farnesylation, and oncogenic/tumor survival pathways. Ongoing preclinical studies are being conducted to determine the efficacy of FTI in combination with other cytotoxic agents in myeloma. These studies should serve as the basis for future clinical trials.

Acknowledgments

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References


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Melissa Alsina, Rafael Fonseca, Edward F. Wilson, A. Nelida Belle, Elvira Gerbino, Tammy Price-Troska, Rose M. Overton, Gregory Ahmann, Laura M. Bruzek, Alex A. Adjei, Scott H. Kaufmann, John J. Wright, Daniel Sullivan, Benjamin Djulbegovic, Alan B. Cantor, Philip R. Greipp, William S. Dalton and Saïd M. Sebti