



Frane Mlinar. *Hidden Cove*. Oil on canvas, 36" × 46". Courtesy of The Weatherburn Gallery, Naples, Florida.

*A farnesyltransferase inhibitor can stabilize patients with advanced multiple myeloma.*

# Farnesyltransferase Inhibitors and Their Role in the Treatment of Multiple Myeloma

*Rodrigo Santucci, MD, Paul A. Mackley, Saïd Sebti, PhD, and Melissa Alsina, MD*

**Background:** *Ras mutations are among the most common oncogene mutations found in multiple myeloma (MM). Patients with mutated Ras are less likely to respond to chemotherapy and have a shortened median survival. Therefore, targeting Ras farnesylation may be a valuable approach to treatment of MM. R115777 (tipifarnib) is a potent farnesyltransferase inhibitor (FTI) presently undergoing phase II/III clinical trials.*

**Methods:** *We reviewed the preclinical and clinical experience of FTIs as antineoplastic agents and describe their potential role in the treatment of MM.*

**Results:** *FTIs are a novel group of agents that selectively inhibit farnesyltransferase, an enzyme responsible for the posttranslational modification of several proteins including Ras. Since Ras is among the most commonly mutated oncogenes associated with cancer, this class of drugs has been evaluated in clinical trials in a diversity of tumors. R115777 has been evaluated in a phase II clinical trial in patients with advanced myeloma and found to be well tolerated. It induced disease stabilization in more than 60% of patients with advanced myeloma.*

**Conclusions:** *The drug selectively targets farnesyltransferase, but this effect did not correlate with disease stabilization, suggesting that these drugs may be targeting a survival pathway independent of Ras processing. Further studies will evaluate the use of FTI in maintenance therapy as well as in combination with other agents in advanced myeloma.*

*From the Department of Interdisciplinary Oncology (PAM, SS, MA) and Drug Discovery Program (SS) at the H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, and the Discipline of Hemato-Oncology (RS) of the ABC Foundation School of Medicine, São Paulo, Brazil.*

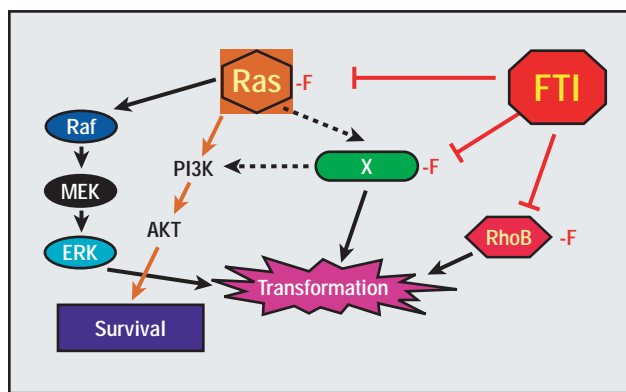
*Submitted July 7, 2003; accepted August 18, 2003.*

*Address reprint requests to Melissa Alsina, MD, H. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, Tampa, FL 33612. E-mail: alsinam@moffitt.usf.edu*

*No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.*

## Introduction

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,<sup>1</sup> drug resistance,<sup>2,3</sup> and bone disease.<sup>4</sup> This understanding has led to the development of new treatments that can not only induce myeloma cell cytotoxicity but also interfere with



Farnesyltransferase inhibitor (FTI) targets.

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 (IL-6).<sup>5</sup> The adhesion of myeloma cells to stromal cells induces IL-6 secretion by the stromal cells, which in turn mediates myeloma cell growth through the activation of signal transduction pathways involved in cell growth and transformation, such as the STAT, AKT and Ras/ERK1-2 pathways.<sup>6,7</sup> These signal transduction pathways downstream from IL-6, represent potential treatment targets in multiple myeloma (MM). Among these, the Ras oncogene is of particular interest as a potential therapeutic target, as Ras mutations represent the most common gene mutation in MM.<sup>8</sup>

Farnesyltransferase inhibitors (FTIs) are a group of drugs that selectively inhibit the enzyme farnesyltransferase (FTase) that is responsible for the transfer of a farnesyl group to Ras and other proteins involved in signaling concerning cell transformation and survival (Figure).<sup>9-11</sup>

We review the reported experience with FTIs as antineoplastic agents and discuss their current and future use in the treatment of MM.

## Farnesyltransferase Inhibitors

FTIs comprise a novel class of antineoplastic agents recently developed to inhibit FTase.<sup>12</sup> While these inhibitors were designed to target Ras, it is evident in many instances that Ras may not be the only target of FTIs. In many tumor cell lines including myeloma, the antitumor activity of FTI does not correlate with mutated Ras status.<sup>13,14</sup> The finding that *K-ras* and *N-ras* can be prenylated by geranylgeranyl transferase also argues against Ras as the dominant target, as preclinical models bearing these mutations are sensitive to FTI treatments.<sup>15</sup> To date, many proteins have been suggested as potential FTI targets.<sup>16</sup> Therefore, in

theory, the inhibition of farnesylation of any of these peptides can explain the antiproliferative effects of FTIs in human tumors.

Despite uncertainty about the true target of FTIs, the agents demonstrate anticancer activity when used as single agents and in combination with cytotoxic chemotherapy. In addition, FTIs synergize with gamma radiation and may play a role in chemoprevention.<sup>17,18</sup> To date, several FTIs have been evaluated in clinical trials, including BMS-214664, L-778,123, R115777, and SCH-66363. Overall, FTIs have been shown to have mild toxicities, including neutropenia, thrombocytopenia, diarrhea, rash, neuropathy, and transaminase elevations. Clinical activity has been demonstrated in breast cancer,<sup>19</sup> myelodysplastic syndrome,<sup>20</sup> chronic myelogenous leukemia, and acute leukemias.<sup>21</sup>

## Ras Oncogene in Myeloma

The Ras oncoprotein is a monomeric membrane-localized G protein signal transducer of 21-kD molecular weight that requires prenyl lipid modification and membrane association for signal transduction activity.<sup>22</sup> This modification involves the covalent addition of either farnesyl (15-carbon) or geranylgeranyl (20-carbon) groups to conserved carboxy terminal cysteine residues of certain proteins. The enzymes that catalyze this modification are FTase and geranylgeranyl transferase, respectively. Mutations in Ras result in constitutive activity that can lead to uncontrolled proliferation and inhibition of apoptosis.<sup>23</sup>

In 1996, Liu et al<sup>24</sup> found Ras mutations in 39% of newly diagnosed myeloma patients as well as a correlation between Ras mutation and shorter survival. Patients with Ras mutations had a median survival of 2.1 years compared with 4.0 years for patients with wild-type Ras. Bezieau et al<sup>25</sup> reported a similar incidence of Ras mutations at diagnosis that increased to 81% at the time of disease relapse. Furthermore, Kalakonda and colleagues<sup>26</sup> reported that *N-ras* 61 mutation-positive cells could be detected in subpopulations of tumor cells in all cases of newly diagnosed myeloma patients. These findings provide further evidence that Ras mutations are the most prevalent oncogenic mutations in MM.

## R115777

R115777 (tipifarnib) is an imidazole-containing heterocyclic compound that is a potent nonpeptidomimetic inhibitor of FTase.<sup>27</sup> The growth of several human tumor cell lines, including those with either

wild-type or mutant Ras, is inhibited with 50% inhibitory concentrations ( $IC_{50}$ s) ranging from 1.7 to 50 nmol/L.<sup>28,29</sup> In vivo bid dosing shows a dose-dependent inhibition of human colon and pancreatic cancer xenografts, with antitumor effects including apoptosis, decrease of proliferation, and antiangiogenesis.<sup>30</sup> In phase I trials, R115777 has been administered at doses up to 1300 mg p.o. b.i.d. for 5 days every 2 weeks without significant toxicities.<sup>31</sup> Two additional phase I studies have investigated dosing for 14 or 21 days followed by 7 days of rest. The dose-limiting toxicity was reversible myelosuppression.<sup>32</sup> The drug has demonstrated clinical activity in phase I and II studies of patients with metastatic breast cancer, myelodysplastic syndrome, and acute myelogenous leukemia.<sup>33-35</sup>

In myeloma, the prenylation inhibitors FTI-277 and geranylgeranyl transferase I inhibitor (GGTI-2166) were shown to induce apoptosis in myeloma cell lines selected for resistance to classic cytotoxics, including doxorubicin and melphalan.<sup>36</sup> Similarly, we and others have shown that R115777 induces a dose- and time-dependent growth inhibition and apoptosis in myeloma cell lines.<sup>37,38</sup>

## Phase II Trial of R115777 in Advanced Multiple Myeloma

We conducted a phase II trial to evaluate the activity and tolerability of R115777 and also to correlate response to inhibition of protein farnesylation and oncogenic/tumor survival pathways in patients with advanced MM.<sup>39</sup> Eligibility criteria included patients that meet criteria for relapsed or refractory myeloma, ECOG performance status <3, normal renal function, and measurable disease. FTI 300 mg given orally b.i.d. was administered for 3 weeks and was repeated every 4 weeks. The dose was to be escalated after 1 cycle to 400 mg b.i.d. in the absence of grade 3 toxicity. Patients were evaluated after 2 cycles and treatment was continued if they had a response, improvement, or stabilization of disease according to modified SWOG criteria for disease response.<sup>40</sup> Forty-three patients entered the study. The median age was 62 years (range = 33 to 82). The patients were all heavily pretreated, with a median of 3.7 chemotherapy regimens prior to entering the study. Fifty-four percent of the patients had prior thalidomide or high-dose chemotherapy and stem cell/bone marrow transplant. On entering the study, half of the patient group was refractory to their most recent treatment. The most common toxicity was fatigue, which occurred in 66% of patients. Other toxicities included diarrhea, nausea, neuropathy, anemia, and thrombocytopenia. Sixty-two percent of the patients had a reduction of the monoclonal protein of

less than 50% consistent with disease stabilization. Treatment with R115777 suppressed FTase but not GGase I activity in bone marrow and peripheral blood mononuclear cells of patients with MM. Similarly, R115777 inhibited the prenylation of the farnesylated protein HDJ-2 in all patients, and it decreased the levels of phosphorylated Akt and STAT3 but not Erk1/2 in bone marrow from patients in whom these oncogenic tumor survival pathways were constitutively activated. Inhibition of farnesylation did not correlate with clinical activity. We conclude that R115777 is tolerable and can induce disease stabilization in patients with MM, and that 300 mg b.i.d. is sufficient to inhibit FTase activity, protein farnesylation, and the oncogenic/tumor survival pathway.

## Current and Future Studies

We learned from this clinical trial that while protein farnesylation was inhibited in all patients, this event did not correlate with clinical activity. Clinical results indicated that R115777 reduced the levels of phosphorylated Akt and STAT3 in bone marrow from patients in whom these tumor survival pathways were constitutively active, and the former correlated with disease stabilization in the limited number of patients examined. The PI 3-kinase/AKT2 pathway has been shown to be a critical target for FTI-induced apoptosis in ovarian cancer cell lines.<sup>41</sup> Therefore, we examined the mechanisms of cytotoxicity of R115777 on myeloma cell lines and its correlation with AKT activity. R115777 inhibited proliferation in all cell lines except MM1 at concentrations <5  $\mu$ m, with RPMI 8226 showing the most sensitivity ( $IC_{50}$   $2 \times 10^{-8}$  M) and U266 and H929 showing a more moderate sensitivity of  $2 \times 10^{-6}$  M and  $4 \times 10^{-7}$  M, respectively. Propidium iodide cell cycle analysis indicated that 8226 and H929 cells accumulate in  $G_2$ -M phase and  $G_1$ - $G_0$  phase, respectively, in a dose-dependent manner. Annexin V-PI analysis indicated a dose-dependent increase in the number of apoptotic cells in all cell lines except MM1s. A dose of 1  $\mu$ m R115777 induced pro-caspase 3 cleavage in 8226 cells, but not in MM1 cells, between 12 and 24 hours after treatment. R115777 induced a dose-dependent pro-caspase 3 cleavage in 8226, U266, and H929 cells within 72 hours. MM1 cells under the same conditions failed to exhibit a similar response after 72 hours of treatment. FTI inhibited AKT phosphorylation in a dose-dependent manner in all MM cell lines examined. The levels of phosphorylated AKT detected correlated with resistance to FTI, with the more resistant cell lines showing higher levels of phosphorylated AKT and incomplete inhibition when treated with FTI.<sup>38</sup> Our data suggest that the AKT tumor survival pathway plays an important role in R115777-induced apoptosis in myeloma.

## Conclusions

FTIs are a new class of agents with significant antimyeloma activity in vitro. In patients, R115777 induced stabilization of disease in 62% of patients with advanced MM. Further clinical studies will examine the clinical activity of this agent in combination with other cytotoxics or as maintenance therapy in patients with MM.

## References

1. Anderson KC. Targeting myeloma cell-host bone marrow interactions. *American Society of Clinical Oncology 2002 Education Book*. 2002:79-486.
2. Damiano JS, Cress AE, Hazlehurst LA, et al. Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. *Blood*. 1999;93:1658-1667.
3. Shain KH, Dalton WS. Cell adhesion is a key determinant in de novo multidrug resistance (MDR): new targets for the prevention of acquired MDR. *Mol Cancer Ther*. 2001;1:69-78.
4. Michigami T, Shimizu N, Williams PJ, et al. Cell-cell contact between marrow stromal cells and myeloma cells via VCAM-1 and alpha(4)beta(1)-integrin enhances production of osteoclast-stimulating activity. *Blood*. 2000;96:1953-1960.
5. Ogata A, Chauhan D, Teoh G, et al. IL-6 triggers cell growth via the Ras-dependent mitogen-activated protein kinase cascade. *J Immunol*. 1997;159:2212-2221.
6. Hideshima T, Nakamura N, Chauhan D, et al. Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma. *Oncogene*. 2001;20:5991-6000.
7. Puthier D, Bataille R, Amiot M. IL-6 up-regulates mcl-1 in human myeloma cells through JAK/STAT rather than Ras/MAP kinase pathway. *Eur J Immunol*. 1999;29:3945-3950.
8. Corradini P, Ladetto M, Voena C, et al. Mutational activation of N- and K-ras oncogenes in plasma cell dyscrasias. *Blood*. 1993;81:2708-2713.
9. Adjei AA. Blocking oncogenic Ras signaling for cancer therapy. *J Natl Cancer Inst*. 2001;93:1062-1074.
10. Dancey JE. Agents targeting ras signaling pathway. *Curr Pharm Des*. 2002;8:2259-2267.
11. Sebti S, Hamilton AD. Inhibitors of prenyl transferases. *Curr Opin Oncol*. 1997;9:557-561.
12. Kohl NE. Farnesyltransferase inhibitors: preclinical development. *Ann NY Acad Sci*. 1999;886:91-102.
13. Feldkamp MM, Lau N, Roncari L, et al. Isotype-specific Ras GTP-levels predict the efficacy of farnesyl transferase inhibitors against human astrocytomas regardless of Ras mutational status. *Cancer Res*. 2001;61:4425-4431.
14. Shi Y, Gera J, Hsu JH, et al. Cytoreductive effects of farnesyl transferase inhibitors on multiple myeloma tumor cells. *Mol Cancer Ther*. 2003;2:563-572.
15. Lobell RB, Omer CA, Abrams MT, et al. Evaluation of farnesyl:protein transferase and geranylgeranyl:protein transferase inhibitors combinations in preclinical models. *Cancer Res*. 2001;61:8758-8768.
16. Kamasani U, Liu AX, Prendergast GC. Genetic response to farnesyltransferase inhibitors: proapoptotic targets of RhoB. *Cancer Biol Ther*. 2003;2:273-280.
17. Lieberman R, Bermejo C, Akaza H, et al. Progress in prostate cancer chemoprevention: modulators of promotion and progression. *Urology*. 2001;58:835-842.
18. Jones HA, Hahn SM, Bernhard E, et al. Ras inhibitors and radiation therapy. *Semin Radiat Oncol*. 2001;11:328-337.
19. Johnston SR, Kelland LR. Farnesyl transferase inhibitors: a novel therapy for breast cancer. *Endocr Relat Cancer*. 2001;8:227-235.
20. Kurzrock R, Kantarjian HM, Cortes JE, et al. Farnesyltransferase inhibitor R115777 in myelodysplastic syndrome: clinical and biologic activities in the phase I setting. *Blood*. 2003. In press.
21. Karp JE, Lancet JE, Kaufmann SH, et al. Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: a phase I clinical-laboratory correlative trial. *Blood*. 2001;97:3361-3369.
22. Barbacid M. Ras genes. *Annu Rev Biochem*. 1987;56:779-827.
23. Zhang FL, Casey PJ. Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem*. 1996;65:241-269.
24. Liu P, Leong T, Quam L, et al. Activating mutations of N- and K-ras in multiple myeloma show different clinical associations: analysis of the Eastern Cooperative Oncology Group Phase III Trial. *Blood*. 1996;88:2699-2706.
25. Bezieau S, Devilder MC, Avet-Loiseau H, et al. High incidence of N and K-Ras activating mutations in multiple myeloma and primary plasma cell leukemia at diagnosis. *Hum Mutat*. 2001;18:212-224.
26. Kalakonda N, Rothwell DG, Scarffe JH, et al. Detection of N-Ras codon 61 mutations in subpopulations of tumor cells in multiple myeloma at presentation. *Blood*. 2001;8:1555-1560.
27. Venet M, End D, Angibaud P. Farnesyl protein transferase inhibitor ZARNESTRA R115777: history of a discovery. *Curr Top Med Chem*. 2003;3:1095-1102.
28. End DW, Smets G, Todd AV, et al. Characterization of the anti-tumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. *Cancer Res*. 2001;61:131-137.
29. Smith V, Rowlands MG, Barrie E, et al. Establishment and characterization of acquired resistance to the farnesyl protein transferase inhibitor R115777 in a human colon cancer cell line. *Clin Cancer Res*. 2002;8:2002-2009.
30. Dempke WC. Farnesyltransferase inhibitors: a novel approach in the treatment of advanced pancreatic carcinomas. *Anti-cancer Res*. 2003;23:813-818.
31. Crul M, de Klerk GJ, Swart M, et al. Phase I clinical and pharmacologic study of chronic oral administration of the farnesyl protein transferase inhibitor R115777 in advanced cancer. *J Clin Oncol*. 2002;20:2726-2735.
32. Adjei AA, Croghan GA, Erlichman C, et al. A phase I trial of the farnesyl protein transferase inhibitor R115777 in combination with gemcitabine and cisplatin in patients with advanced cancer. *Clin Cancer Res*. 2003;9:2520-2526.
33. Johnston SR, Hickish T, Ellis P, et al. Phase II study of the efficacy and tolerability of two dosing regimens of the farnesyl transferase inhibitor, R115777, in advanced breast cancer. *J Clin Oncol*. 2003;21:2492-2499.
34. Adjei AA, Mauer A, Bruzek L, et al. Phase II study of the farnesyl transferase inhibitor R115777 in patients with advanced non-small-cell lung cancer. *J Clin Oncol*. 2003;21:1760-1766.
35. Cohen SJ, Ho L, Ranganathan S, et al. Phase II and pharmacodynamic study of the farnesyltransferase inhibitor R115777 as initial therapy in patients with metastatic pancreatic adenocarcinoma. *J Clin Oncol*. 2003;21:1301-1306.
36. Bolick SC, Landowski TH, Boulware D, et al. The farnesyl transferase inhibitor, FTI-277, inhibits growth and induces apoptosis in drug-resistant myeloma tumor cells. *Leukemia*. 2003;17:451-457.
37. Ochiai N, Uchida R, Fuchida SI, et al. Effect of farnesyl transferase inhibitor R115777 (Zarnestra) on the growth of fresh and cloned myeloma cells in vitro. *Blood*. 2003;July 3. Epub ahead of print.
38. Mackley PA, Shain KH, Dalton WS, et al. Farnesyl transferase inhibitor R115777 decreases Akt phosphorylation in multiple myeloma cell lines and its apoptotic effects correlate with phosphoAKT expression levels. *Blood*. 2002;3198. Abstract.
39. Alsina M, Fonseca R, Wilson EE, et al. The farnesyl transferase inhibitor Zarnestra is well tolerated, induces stabilization of disease and inhibits farnesylation and oncogenic/tumor survival pathways in patients with advanced multiple myeloma. *Blood*. 2003. Submitted.
40. Salmon SE, Crowley JJ, Grogan TM, et al. Combination chemotherapy, glucocorticoids, and interferon alfa in the treatment of multiple myeloma: a Southwest Oncology Group study. *J Clin Oncol*. 1994;12:2405-2414.
41. Jiang K, Coppola D, Crespo NC, et al. The phosphoinositide 3-OH kinase/AKT2 pathway as a critical target for farnesyltransferase inhibitor-induced apoptosis. *Mol Cell Biol*. 2000;20:139-148.