Apoptotic and Cytostatic Farnesyltransferase Inhibitors Have Distinct Pharmacology and Efficacy Profiles in Tumor Models

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ABSTRACT

BMS-214662 and BMS-225975 are tetrahydrobenzodiazepine-based farnesyltransferase inhibitors (FTIs) that have nearly identical structures and very similar pharmacological profiles associated with farnesyltransferase (FT) inhibition. Despite their similar activity against FT in vitro and in cells, these compounds differ dramatically in their apoptotic potency and tumor-regressing activity in vivo. BMS-214662 is the most potent apoptotic FTI known and exhibits curative responses in mice bearing a variety of staged human tumor xenografts such as HCT-116 human colon tumor. By contrast, BMS-225975 does not cause tumor regression and at best causes partial tumor growth inhibition in staged HCT-116 human colon tumor xenografts. Lack of tumor regression activity in BMS-225975 was attributable to its relatively weak apoptotic potency, not to poor cell permeability or pharmacokinetics. Both compounds were equally effective in inhibiting Ras processing and causing accumulation of a variety of nonfarnesylated substrates of FT in HCT-116 cells. Because BMS-225975 has poor apoptotic activity compared with BMS-214662 but inhibits FT to the same extent as BMS-214662, it is very unlikely that FT inhibition alone can account for the apoptotic potency of BMS-214662. Clearly distinct patterns of sensitivities in a cell line panel were obtained for the apoptotic FTI BMS-214662 and the cytostatic FTI BMS-225975. Activation of the c-Jun-NH2-terminal kinase pathway was readily observed with BMS-214662 but not with BMS-225975. We developed a highly sensitive San-1 murine xenograft tumor model that is particularly useful for evaluating the in vivo activity of cytostatic FTIs such as BMS-225975.

INTRODUCTION

Farnesyltransferase inhibitors (FTIs) were originally developed as agents to inhibit tumor growth, by inhibiting the farnesylation, processing, and membrane anchoring of Ras proteins in cells (1–5). Over the past 13 years, a number of structurally diverse FTIs have been identified and studied as potential anticancer agents (6–8). Several FTIs such as R-115777, SCH-66336, L-778123, and BMS-214662 have advanced into clinical development.

In general, all FTIs selectively affect the growth of Ras-transformed cells and exhibit antitumor activity in preclinical animal models (7, 9, 10). Recent studies portray a highly complex nature to the biology and preclinical antitumor activity of FTIs (11). In FTI-treated cells, K- and N-Ras proteins have been shown to undergo geranylgeranylation catalyzed by geranylgeranyltransferase I and potentially remain functional (12, 13). Despite this, FTIs, in general, demonstrate in vitro and in vivo activity against tumor cells bearing K-Ras mutations (13, 14). Moreover, no clear correlation between Ras mutation status and FTI sensitivity has been observed in studies conducted by several investigators (13–15). Thus, the observed antitumor properties of FTIs are not solely due to Ras inhibition but may reflect inhibition of farnesylation of other proteins (16). Prenylated proteins such as RhoB and centromere-associated CENP-E and -F have been proposed as the molecular targets of FTIs in all or some tumor cells (11, 17). However, there remains no consensus as to the relevant target(s) of FTIs that can satisfactorily account for the pharmacology of FTIs.

Apoptosis induction by cancer chemotherapeutic drugs is a highly sought and desired characteristic. Originally, FTIs were anticipated to be cytostatic and therefore clinically useful as chronically administered agents (18, 19). Proapoptotic or apoptotic properties in FTIs were suggested as a potential mechanism to explain tumor regression induced by the FTI L-739749 in v-H-Ras transgenic mice (20). Subsequently, a variety of other FTIs or agents that block isoprenylation of proteins by indirect mechanisms were shown to induce apoptosis in Ras-transformed rodent fibroblasts and in human tumor cells (21–23). It is not clear at present if and how FTI-induced apoptosis is related to farnesyltransferase (FT) inhibition in cells because no systematic attempt has been made to conduct studies at concentrations that are relevant to FT inhibition and apoptosis.

BMS-214662 and BMS-225975 [(R)-7-cyano-2,3,4,5-tetrahydro-1-(1H-1-methyl-imidazol-5-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1H-1,4-benzodiazepine hydrochloride] are tetrahydrobenzodiazepine-based FTIs that differ structurally by a single methyl group. The in vitro potencies of these compounds against FT and geranylgeranyltransferase I are remarkably similar, and yet they display dramatically different activity in apoptosis induction and in vivo antitumor activity. Using these two compounds as tools, we developed a pharmacological profile for highly apoptotic and cytostatic FTIs. We also demonstrate a disconnect between Ras or FT inhibition and apoptosis induction by tetrahydrobenzodiazepine FTIs in tumor cells.

MATERIALS AND METHODS

Synthesis of BMS-225975. A mixture of [(R)-7-cyano-2,3,4,5-tetrahydro-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1H-1,4-benzodiazepine (150 mg, 0.366 mmol), 1-methyl-5-formylimidazole (121 mg, 1.10 mmol), and 200 mg of 3A molecular sieves in 2 ml of 3:1 DCE/AcOH was heated at 60°C. At 1, 4, 7, and 10 h, aliquots of sodium triacetoxycoborohydride (116 mg, 0.549 mmol) were added. At 3, 6, and 9 h, aliquots of aldehyde (91 mg, 0.946 mmol) were added. Acetic acid (1 ml) was also added at 9 h. After the last addition of hydride, the mixture was stirred at 60°C for 2 h, diluted with 5 ml of methanol, filtered, and concentrated under vacuum. The residue was diluted with 100 ml of EtOAc and washed with 1N NaOH (3 × 50 ml), and brine. The organic layer was dried over Na2SO4 and filtered and concentrated under vacuum. The residue was purified by reverse-phase preparative high-performance liquid chromatography (gradient of aqueous methanol with 0.1% trifluoroacetic acid), and the appropriate fractions were concentrated. The residue was dissolved in 1 m HCl (3 × 5 ml) and concentrated under vacuum. The residue was dissolved in minimal acetonitrile, diluted with water, and freeze-dried to provide 60 mg of BMS-225975 as a white solid ([M+H]+ 504; analysis calculated for C27H24N2O4S2·1.50 HCl·0.66 H2O (calculated: C, 54.77; H, 4.92; N, 12.28; found: C, 54.77; H, 4.92; N, 12.25)].

Drug Preparation. For in vitro studies, the hydrochloride salts of BMS-214662 and BMS-225975 were dissolved in DMSO with dilutions made using either water or RPMI 1640 (Gibco) plus 10% fetal bovine serum (Gibco). The compounds were dissolved in ethanol followed by dilution with water to a final

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ethanol concentration of 10% for many in vivo studies. This latter vehicle was used for all oral (p.o.) administrations.

**Pharmacology Assays.** The assays for prenyltransferase, reversion, soft agar growth, and H-Ras processing inhibition were carried out as described previously (24, 25). Apoptosis was measured using the cell death detection ELISAplus kit (nucleosomal DNA ELISA assay) supplied by Boehringer Mannheim.

**Cell Lines and Culture.** The Rat1CVLS cell line has been described previously (24) as a RC-165 cells transformed by human genomic K-Ras4B DNA (which contains the human K-ras4B minigene with a G418 selectable marker with Cys-12 mutation). MCF-10A/Ras cells were obtained by cotransfecting MCF-10A cells with a plasmid containing the neomycin selectable marker with Cys-12 mutation. MCF-10A/Ras cells were obtained by cotransfecting MCF-10A cells with a plasmid containing the neomycin selectable marker with Cys-12 mutation)

**Detection of FTI-Affected Substrates.** HCT-116 tumor cells were plated in a 6-well dish at 1–2 × 10⁶ cells/well in 3 ml of culture medium (DMEM + 10% fetal bovine serum) and allowed to attach for 24 h at 37°C. Compounds were added at the indicated concentrations, and the cells were incubated for 24 h at 37°C/7% CO₂. After removal of the medium and two rinses with ice-cold PBS, cells were harvested by scraping and centrifugation. Cell pellets were washed again in ice-cold PBS, extracts were prepared in 70 μl of NP40 lysis buffer [20 mM Tris-HCl (pH 7.4), 1 mM DTT, 1% protease inhibitor mixture, and 1% NP40]. Samples were clarified by centrifugation, and 15 μl were used for in vitro farnesylation assay using excess human recombinant FT and [3H]farnesylo phosphosphate (FPP) under standard assay conditions (15). After analysis of samples by SDS-PAGE on 12.5% gels, the left gels were subjected to fluorography and autoradiography. The right gels were subjected to fluorography and autoradiography.

**Activation of c-Jun-NH²-Terminal Kinase (JNK).** HCT-116 tumor cells were plated in a 6-well dish at 0.9 × 10⁶ cells/well in 3 ml of culture medium (DMEM + 10% fetal bovine serum) and allowed to attach for 24 h at 37°C. Compounds were added at the indicated concentrations, and the cells were incubated for 24 h at 37°C/7% CO₂. After removal of the medium and two quick rinses with ice-cold PBS, cells were harvested by scraping with 150 μl of TTG lysis buffer [20 mM Tris-HCl (pH 7.7), 1% Triton X-100, 5% glycerol, 120 mM NaCl, 0.2 mM EDTA, 1 mM sodium vanadate, and 40 μM ammonium molybdate]. Samples were clarified by centrifugation, and 15 μl were used for SDS-PAGE analysis. Antibodies specific for phospho-JNK and JNK were from Cell Signaling.

**Mice.** CDF1 mice and BALB/c background athymic (nude) female mice approximately 5 weeks of age were purchased from Harlan Sprague Dawley (Indianapolis, IN). SENCAR mice were obtained from the National Cancer Institute. Animals were provided with food and water ad libitum. All studies involving these animals were conducted in accordance with NIH and Bristol-Myers Squibb Company animal care and use guidelines.

**Tumors and Efficacy Testing.** The human colon tumor line HCT-116, passed s.c. in vivo at approximately 2–3-week intervals, was used. The San-1 murine tumor was also passed s.c. at 2-week intervals. The following Ras mutations are known to exist and/or were confirmed at Bristol-Myers Squibb Company in these tumors: K-Ras (HCT-116); and H-Ras (San-1). Detailed descriptions of efficacy testing and assessment have been reported previously (15).

**San-1 Tumor Model.** The multistage model involving initiation and promotion/progression resulting in ras oncogene mutations was used (26). A single topical application of 5 μg of 7,12-dimethylbenz(a)anthracene in 0.2 ml of acetone was made to several SENCAR mice. Two weeks later, the site of 7,12-dimethylbenz(a)anthracene application was treated with 1 μg of 12-O-tetradecanoylphorbol-13-acetate twice per week, and the treatment was continued for 16 weeks. When 12-O-tetradecanoylphorbol-13-acetate treatment was stopped in 16 weeks, papillomas appeared. Several papillomas converted to squamous cell carcinomas in another 14 weeks. Five of the six carcinomas showed A to G transversion in the second position of codon 61 of the H-ras gene. Two carcinomas with the mutated H-ras gene were transplanted into nude mice and propagated for several weeks with repeated passage through nude mice until a stabilized growth was observed. One of these better growing was named San-1 and was verified to contain the codon 61 H-ras mutation.

**Pharmacokinetics.** Limited oral pharmacokinetics of BMS-214662 and BMS-225975 were performed in CDF1 mice. Mice were given a single bolus dose (200 mg/kg) of BMS-214662 or BMS-225975 by oral gavage in 10% ethanol solution. Blood samples were collected over a period of 8 h, and the concentration of compounds was determined by LC/UV assay.

**In Vitro Cytotoxicity Assay.** In vitro cytotoxicity was assessed by a vital dye assay using a tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2H-tetrazolium (inner salt), as described previously (15). The IC₅₀ values for FTIs were calculated and expressed graphically as a mean bar graph on a log scale for each cell line exposed to the compound. The difference between the mean log IC₅₀ value and each individual cell line’s log IC₅₀ value was calculated and plotted. Bars that project to the right represent cells that are more sensitive than the mean IC₅₀ for all of the cell lines, whereas bars that project to the left represent cell lines that are less sensitive (more resistant) than the mean IC₅₀ (Fig. 7).

**RESULTS**

**In Vitro Activity.** BMS-214662 and BMS-225975, tetrahydrobenzodiazepine-based FTIs, are structurally identical except that the hydrogen on the imidazole of BMS-214662 is replaced in BMS-225975 with a methyl group on the tau nitrogen (Fig. 1). Whereas the hydroxybenzodiazepine-based FTIs, are structurally identical except that the hydrogen on the imidazole of BMS-214662 is replaced in BMS-225975 with a methyl group on the tau nitrogen (Fig. 1). Whereas the positions of HBZFTI-Affected Substrates.

**Activation of c-Jun-NH²-Terminal Kinase (JNK).** HCT-116 tumor cells were plated in a 6-well dish at 1–2 × 10⁶ cells/well in 3 ml of culture medium (DMEM + 10% fetal bovine serum) and allowed to attach for 24 h at 37°C. Compounds were added at the indicated concentrations, and the cells were incubated for 24 h at 37°C/7% CO₂. After removal of the medium and two rinses with ice-cold PBS, cells were harvested by scraping and centrifugation. Cell pellets were washed again in ice-cold PBS, extracts were prepared in 70 μl of NP40 lysis buffer [20 mM Tris-HCl (pH 7.4), 1 mM DTT, 1% protease inhibitor mixture, and 1% NP40]. Samples were clarified by centrifugation, and 15 μl were used for in vitro farnesylation assay using excess human recombinant FT and [3H]farnesyl phosphosphate (FPP) under standard assay conditions (15). After analysis of samples by SDS-PAGE on 12.5% gels, the gels were subjected to fluorography and autoradiography.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Protein substrate</th>
<th>Prenyl donor</th>
<th>Mean IC₅₀ (nm)</th>
<th>Mean IC₅₀ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT</td>
<td>H-Ras</td>
<td>FPP</td>
<td>1.3</td>
<td>18</td>
</tr>
<tr>
<td>FT</td>
<td>K-Ras</td>
<td>FPP</td>
<td>8.4</td>
<td>108</td>
</tr>
<tr>
<td>GTT</td>
<td>K-Ras</td>
<td>GGPP</td>
<td>1,900</td>
<td>17,700</td>
</tr>
</tbody>
</table>

* FT, farnesyltransferase; GTTI, geranylgeranyltransferase I; FPP, farnesyl phosphosphate; GGPP, geranylgeranyl phosphophosphate.
transformation phenotype or the inhibition of soft agar growth assays (Table 2).

**Antitumor Activity in Vivo.** We have previously described broad spectrum antitumor activity of BMS-214662 in human tumor xenografts of diverse histological sources (15). Curative activity against established tumors was demonstrated by parenteral and oral routes of drug administration. We expected to see similar antitumor activity for the two FTIs. One noteworthy marker that is activated in response to apoptotic potency between BMS-214662 and BMS-225975 was observed in both MCF-10A and the same cell line transformed with Ras (MCF-10A/Ras), thus excluding a role for Ras transformation in the apoptosis induction by BMS-214662 (Fig. 4). The relatively inferior apoptotic potency of BMS-225975 is not attributable to lack of cell permeability because it is more potent than BMS-214662 in the Ras transformation reversion assay and soft agar growth assays (Table 2).

**Activation of the JNK Pathway.** To account for the apoptotic activity inherent in BMS-214662 but not BMS-225975, we explored several biochemical markers in HCT-116 cell extracts treated with the two FTIs. One noteworthy marker that is activated in response to BMS-214662 treatment but not BMS-225975 is the JNK pathway (Fig. 5). BMS-214662 induced activation of phospho-JNK in a concentration-dependent manner. In contrast, BMS-225975, despite being a very close analog, produced very little activation at high concentrations.

**Inhibition of Ras and Other Protein Substrate Farnesylation.** Inhibition of H-Ras processing and accumulation of unmodified H-Ras proteins occur readily in HCT-116 human colon tumor cells, and the disappearance of membrane-bound Ras was nearly complete in 24 h with as little as 100 nM BMS-214662 (15). BMS-225975 was equally effective in inhibiting H-Ras processing, and complete inhibition occurred with 80 nM BMS-225975 in 24 h (data not shown). These results establish that complete inhibition of Ras processing is not sufficient to result in apoptosis.

To determine whether overall farnesylation of various cell proteins is differentially affected by the two compounds, accounting for the difference in apoptotic potencies, we identified farnesylated proteins in cellular extracts from HCT-116 cells treated with BMS-214662 and BMS-225975. The farnesylated proteins were identified by labeling with [3H]FPP and excess human recombinant FT. The results, presented in Fig. 6, show that global farnesylation was af-

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### Table 2. Cell activity of BMS-214662 and BMS-225975

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Mutation</th>
<th>Assay used</th>
<th>IC50 (μM)</th>
<th>IC90 (μM)</th>
<th>IC50 (μM)</th>
<th>IC90 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratl-CVLS</td>
<td>H-Ras</td>
<td>SAG* assay</td>
<td>0.1</td>
<td>0.3</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>NIH3T3 (44-911)</td>
<td>H-Ras</td>
<td>SAG* assay</td>
<td>0.025</td>
<td>0.15</td>
<td>0.008</td>
<td>0.04</td>
</tr>
<tr>
<td>Ratl (RC-165)</td>
<td>K-Ras</td>
<td>SAG assay</td>
<td>0.3</td>
<td>0.62</td>
<td>0.1</td>
<td>0.55</td>
</tr>
<tr>
<td>HCT-116</td>
<td>K-Ras</td>
<td>SAG assay</td>
<td>0.06</td>
<td>0.15</td>
<td>0.03</td>
<td>0.15</td>
</tr>
</tbody>
</table>

* SAG, soft agar growth.
These results show that the overall farnesylation is affected maximally and essentially in a similar manner by both compounds at 200 nM. These results show that the overall farnesylation is similar in cells treated with both compounds and maximal at concentrations that are apoptotic in the case of BMS-214662 and with no significant apoptosis in the case of BMS-225975. As the concentrations were increased to 1 μM, no further increase in labeling intensity was observed, suggesting that maximal inhibition of FT in HCT-116 cells does not require more than 200 nM of these FTIs.

**Cytostasis or Cytotoxicity against a Tumor Cell Line Panel.** To further differentiate the cellular pharmacology of the two FTIs, the compounds were tested in a panel of cell lines consisting of diverse human and mouse tumor cells as well as a bovine endothelial cell line. The data shown in Fig. 7 are expressed as IC_{50} values for each cell line in a bar graph. The individual IC_{50} values and the mean bar graph pattern illustrate that both compounds have robust cell selectivity against a wide variety of tumor cell types. There was a >32-fold and >120-fold difference between the most sensitive and most resistant cell lines for BMS-214662 and BMS-225975, respectively.

Particularly sensitive to BMS-214662 but not to BMS-225975 were human tumor lines OVCAR-3 (ovarian cancer), MCF-7 (breast cancer), LNCAP (prostate cancer), HCT-116 (colon cancer), and A431 (squamous cell carcinoma). Conversely, both mouse cell lines, M109 (lung carcinoma) and MLF (fibroblast cell line), were relatively resistant to BMS-214662 yet somewhat sensitive to BMS-225975.

HCT-116/VM46 colon tumor cells overexpress P-glycoprotein, a drug efflux pump, and are resistant to a number of lipophilic anticancer agents, such as paclitaxel, VP-16, and doxorubicin. The HCT-116/VM46 cells were 4-fold resistant to BMS-214662 relative to the parental HCT-116 cells. Such resistance was not observed for BMS-225975. These results suggest that BMS-214662 but not BMS-225975 may be a weak substrate for P-glycoprotein. These data demonstrate that BMS-225975 inhibits proliferation of a variety of cell lines, yet the profile of cell line sensitivity to this compound is distinct from that for BMS-214662.

**Sensitivity of the San-1 Mouse Tumor Model.** Murine cell lines such as NIH3T3 and Rat1 cells transformed by oncogenic Ras have been used by us and others to demonstrate antitumor activity of FTIs (10, 27). The two FTIs BMS-214662 and BMS-225975 were tested against modestly staged s.c. NIH3T3/Ras (Leu-61) tumors in nude mice. Both compounds were inactive at tolerated doses administered p.o. twice a day for 15 days; however, at a LD_{50} exposure level (300 mg/kg/administration), BMS-225975 did produce 1.1 log cell kill (LCK). It is quite possible that fewer treatments with BMS-225975 would have showed some activity. Lower doses of BMS-225975, however, were ineffective (data not shown). Similar results were obtained using Rat1 cells transformed by oncogenic Ras. Thus, cytostatic FTIs with potent growth inhibition but modest cytotoxicity such as BMS-225975 fail to demonstrate significant antitumor activity in xenograft tumor models. This may be due to the heterogeneity in genetic mutations that is believed to be common in most of these tumor cells and to their ability to depend on other signaling pathways for proliferation or survival. To better assess the potential antitumor efficacy of FTIs, we developed a murine tumor model that is likely to be dependent on Ras signaling.

The San-1 model originated from a squamous cell carcinoma formed in a SENCAR mouse that had been initiated with 7,12-dimethylbenz(a)anthracene and promoted with 12-O-tetradecanoyl-phorbol-13-acetate (26). Sequence analyses showed an A→T trans-
version in the second position of codon 61 of the Ha-ras gene (data not shown). If the H-ras mutation is the primary genetic defect driving uncontrolled growth of the San-1 tumor, the model can be used to characterize compounds that mediate their antitumor activity through inhibition of H-Ras farnesylation. San-1 tumors were implanted s.c. as fragments in nude mice and staged to 100 mg before drug treatment. Given in a twice-daily dosing regimen at 100 mg/kg, BMS-225975 showed significant inhibition of tumor growth in the course of treatment (Fig. 8A). At a dose of 200 mg/kg BMS-225975, the tumors showed a partial regression, but they resumed growth on the cessation of dosing. By contrast, BMS-214662 showed inferior activity in the San-1 model, yielding only marginal activity at the top dose of 300 mg/kg (Fig. 8B). Interestingly, cytotoxic drugs currently used in the clinic such as paclitaxel, cisplatin, and Adriamycin are only marginally active in the San-1 tumor model (data not shown).

**Sensitivity of San-1 Tumor Cells.** To study the reasons for the remarkable difference in the efficacy of the two compounds in the San-1 tumor model, San-1 tumor cells were tested for in vitro sensitivity to BMS-214662 and BMS-225975 using cell growth assays. San-1 tumor cells were very sensitive to growth inhibition by BMS-225975. Concentrations as low as 2.5 nM caused 20–70% growth inhibition in a 5-day growth assay. Similar concentrations of BMS-214662 did not produce such pronounced growth inhibition, causing a maximum of 20% inhibition.

**DISCUSSION**

We reported previously that the FTI BMS-214662 showed remarkable antitumor activity in a significant number of tumor xenograft models, producing curative efficacy in many instances (15). The antitumor efficacy may be attributed, at least in part, to the potency with which BMS-214662 induces apoptosis. What had not been determined was whether the proapoptotic activity was a direct consequence of FT inhibition, or if off-target activity is involved. This report describes the results of our first attempt at understanding the role of FT inhibition in apoptotic induction. We used a pair of FTIs with remarkably similar structure to profile the in vitro, cell, and in vivo properties associated with the tetrahydrobenzodiazepine-based class of FTIs. BMS-214662 and BMS-225975 have single-digit nanomolar FT inhibitory potency and are >1000-fold selective for FT over geranylgeranyltransferase I. BMS-225975 is 2–5-fold more potent than BMS-214662 in terms of whole cell activities associated with FT inhibition such as reversion of the Ras-transformed phenotype, soft agar growth inhibition, and inhibition of Ras processing. Despite having oral pharmacokinetic properties that are comparable with those exhibited by BMS-214662, BMS-225975 showed little efficacy against HCT-116 human colon tumor xenografts, whereas BMS-214662 produced curative antitumor responses. Because of the close FTI pharmacological profile exhibited by these two structurally similar compounds, we were able to demarcate FTI-associated activities...
from other properties inherent to the molecules because of off-target activity, particularly at very high concentrations.

The observation that inhibitors of FT or agents that block isoprenylation of proteins by indirect mechanisms induce apoptosis in Ras-transformed rodent fibroblasts and in human tumor cells has been widely reported by others (20–23, 28–32). Because most of these observations were made at concentrations much higher than those required to completely shut down FT, how and whether FT inhibition is related to apoptosis are not clear. Our results with two closely related FTIs optimized for FT potency through extensive structure activity studies are the first attempt to answer the question and were clearly successful in separating FT inhibition in cells from apoptosis. Whereas it is arguable whether these results are applicable to other FTIs and their reported apoptotic activities, they certainly separate apoptotic activity from FT inhibition inherent in tetrahydrobenzodiazepine-based FTIs.

BMS-214662, with its potent tumor regression and curative activity, differs sharply from other FTIs such as SCH66363, L-744832, R115777, BIM-46228, FTI-277, B-956, and FTI-2153 that act as cytostatic drugs and produce noncurative regressions in transgenic tumor models or selected human tumor xenografts (14, 27, 33–39). Our current results reiterate this by demonstrating that BMS-225975, a remarkably close structural analog of BMS-214662, behaves precisely like many other cytostatic FTIs showing potent inhibition of cell proliferation in vitro and tumor growth in vivo. In contrast, BMS-214662 readily induces apoptosis in cultured cells and is highly efficacious in multiple tumor models, producing tumor regression and curative responses (15). Together, these observations suggest that whereas FT inhibition is sufficient to result in inhibition of tumor growth, the intrinsic proapoptotic characteristics of BMS-214662 appear to be responsible for its outstanding antitumor efficacy.

Lack of in vivo antitumor activity for BMS-214662 against several murine tumors reported previously (15) and the San-1 murine tumor described here is rather perplexing and remains unexplained. We previously excluded potential possibilities such as differential potency against murine FT versus human FT or varying FT enzyme activity levels in resistant cells versus sensitive cells as the sole basis for lack of activity in murine tumors for BMS-214662 (15). It is conceivable that the slightly better cell potency and drug exposure achieved with BMS-225975 may partially account for its in vivo antitumor activity. With respect to the lack of efficacy for BMS-214662 despite its ability to inhibit H-Ras farnesylation, an intriguing question is whether or not the additional activity of the compound, which produces apoptosis, may be protective against FTI-induced growth arrest in San-1 cells. In this case, the tumor model is unsuitable for profiling apoptotic FTIs. Nevertheless, we find the San-1 tumor model to be quite sensitive to various classes of cytostatic FTIs including BMS-191563, a CAAX box-based peptidomimetic analog (40).

The distinct pattern of cytotoxicity results observed for the apoptotic FTI BMS-214662 and the cytostatic FTI BMS-225975 further illustrates the unique mechanism of action for these compounds. The patterns of bar charts depicting IC₅₀ values in a large panel of cell lines are often useful for identifying compounds with a similar mechanism of action or to ascertain unique mechanisms of action in similar compounds (41). The patterns of several apoptotic tetrahydrobenzodiazepine-based FTIs were found to be identical to that of BMS-214662, and these compounds were active in vitro against HCT-116 human colon tumor xenografts. Cytostatic FTIs in the tetrahydrobenzodiazepine series exhibited patterns similar to that shown for BMS-225975, and these compounds were much less apoptotic and caused only inhibition of proliferation in vitro and tumor growth inhibition in vivo. In addition, the patterns of sensitivity in the cell line panel for several N-H/N-Me tetrahydrobenzodiazepine-based FTI pairs were very consistent; the pattern of the N-H analogs was similar to that of BMS-214662, whereas the N-Me analogs displayed a pattern similar to BMS-225975 (data not shown). We also evaluated the patterns of a wide range of chemotherapeutic drugs known to induce apoptosis, including etoposide, doxorubicin, cisplatin, paclitaxel, camptothecin, gemcitabine, and 5-fluorouracil and compared them with selectivity patterns exhibited by BMS-214662 and BMS-225975. No patterns similar to either FTI were observed.

The differential apoptotic potency of the two FTIs was also manifested by the difference in activation of JNK signaling. JNK activation has been associated with apoptotic induction (42). Thus, the observation that BMS-214662, but not BMS-225975, induced JNK activation is consistent with differential activity of these compounds in inducing apoptosis. Activation of the JNK pathway is likely to be unrelated to FT inhibition because the cytostatic FTI BMS-225975 did not cause JNK activation. Another apoptotic FTI, farnesylamine, which induces apoptosis in human pancreatic tumor cells, was reported to induce sustained activation of JNK; however, this effect is not likely due to FT inhibition because FTI-277 did not cause JNK activation (43). Another FTI, L-744832 which induces apoptosis under low serum conditions, was also shown to cause JNK activation (44). Contrary to these observations of JNK activation in tumor cells, FT inhibition was shown to abrogate lipopolysaccharide-induced JNK activation in human peripheral blood monocytes (45).

Rodent cells transformed by Ras and human tumors in xenograft form have been used extensively to generate in vivo antitumor activity profiles of FTIs by us and others (10, 14, 15, 27, 34, 38). Our overall experience has been that established xenografts of H-Ras-transformed NIH3T3 or Rat1 tumors, mutated H-ras-containing EJ-1 human bladder tumor, and mutated K-ras-containing HCT-116 human colon tumor are relatively insensitive to FTIs, in particular, cytostatic FTIs. Thus, these models are suitable for differentiating between FTIs with demonstrable antitumor activity but are not sensitive enough for structure activity relationship purposes and compound drug property optimization. Hence, we developed the San-1 tumor model and showed here that it is highly sensitive to FTIs both at the tumor level in vivo and in vitro cell culture.

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REFERENCES

Correction

In the article on BMS-225975, a cytostatic farnesyltransferase inhibitor in the June 1, 2004 issue of *Cancer Research* (1), Figure 6 is incorrect. The correct figure is below.


![Figure 6](image_url)

Fig. 6. Detection of farnesyltransferase inhibitor-affected substrates by [3H]FPP labeling using exogenous farnesyltransferase. HCT-116 cells were treated with compounds at the indicated concentrations for 24 h, and detergent cell extracts were prepared. The presence of unfarnesylated proteins of various relative molecular weights in the extracts was determined as described in “Materials and Methods.”
Apoptotic and Cytostatic Farnesyltransferase Inhibitors Have Distinct Pharmacology and Efficacy Profiles in Tumor Models


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