Featured Article

Phase I Clinical Trial of the Farnesyltransferase Inhibitor BMS-214662 Given as a 1-Hour Intravenous Infusion in Patients with Advanced Solid Tumors

David P. Ryan, Joseph P. Eder, Jr., Thomas Puchalski, Michael V. Seiden, Thomas J. Lynch, Charles S. Fuchs, Philip C. Amrein, Darrell Sonnichsen, Jeffrey G. Supko, and Jeffrey W. Clark

1Massachusetts General Hospital and 2Dana-Farber Cancer Institute, Dana-Farber/Harvard Cancer Center, Harvard Medical School, Boston, Massachusetts, and 3Bristol-Meyers Squibb, Princeton, New Jersey

Abstract

Purpose: BMS-214662 is a nonnosophore benzodiazepine derivative that exhibits broad spectrum cytotoxicity against human solid tumor cell lines and potently inhibits farnesylation of the H-ras and K-ras oncogenic proteins. This report describes the initial Phase I clinical trial of the compound. The main objective of the study was to determine the dose-limiting toxicities and the maximum tolerated dose of BMS-214662 when administered as a single dose i.v. over 1 h every 21 days to patients with advanced solid tumors.

Experimental Design: Patients with advanced solid tumors and adequate organ function were eligible for the study. The dose was escalated according to a modified Fibonacci schedule after evaluating groups of at least three patients for toxicity during the first cycle of therapy at each dose level. Pharmacokinetic and pharmacodynamic studies were performed after administration of the two initial doses.

Results: The dose of BMS-214662 was escalated from 36 to 225 mg/m² through 5 intermediate dose levels in a total of 44 patients. Dose-limiting toxicities occurred in 3 of the 13 (23%) patients during the first cycle of treatment with 225 mg/m², consisting of grade 3 nausea/vomiting in 2 patients and grade 3 diarrhea in another patient. In addition, four of these patients experienced reversible grade 3 transaminitis, which was not considered to be dose-limiting. At the recommended dose for Phase II studies, 200 mg/m², the most common side effects were reversible transaminitis, nausea, and vomiting. Although there were no objective responses, one patient with pancreatic cancer continues to receive treatment more than 3.5 years after entering the study. BMS-214662 exhibited linear pharmacokinetics and had a mean biological half-life of 1.55 ± 0.27 h and a total body clearance of 21.8 ± 10.8 liters/h/m², with a low apparent volume of distribution at steady state (31.5 ± 12.9 liters/m²).

In patients treated with the recommended Phase II dose, the mean maximum plasma concentration of the drug was 6.57 ± 2.94 µg/ml, and farnesyltransferase activity in peripheral blood mononuclear cells decreased to a nadir of 10.5 ± 6.4% of baseline at the end of the infusion but fully recovered within 24 h.

Conclusions: BMS-214662 can be delivered safely as a single 1-h i.v. infusion at a dose that results in pronounced inhibition of farnesyltransferase activity in peripheral blood mononuclear cells. However, the duration of enzyme inhibition was transient, recovering in parallel with the decline in plasma concentrations of this rapidly eliminated drug. Because indications of anticancer activity were observed in several patients, further optimization of the administration schedule for this promising new compound is warranted.

Introduction

Ras is an oncogene that represents a particularly attractive target for anticancer therapies because activating mutations of the ras genes are among the more common genetic aberrations known in human malignancies. The proteins encoded by the ras genes are guanine nucleotide-binding proteins that associate with the inner plasma membrane and transduce external signals to the interior of the cell. They are among the limited set of known proteins that undergo posttranslational farnesylation, a reaction catalyzed by the cytosolic enzyme farnesyltransferase [FT (1)]. This modification allows ras proteins to anchor to the inner surface of the plasma membrane and is required for normal functions in signal transduction. Consequently, inhibiting farnesylation severely impairs the function of ras proteins. A variety of low molecular weight compounds that inhibit ras farnesylation have been identified, including peptidomimetics of the CAAX COOH terminus of ras, farnesyl diphosphate analogs that function as competitive inhibitors of FT, natural products, and synthetic nonpeptidomimetic compounds (2). Among these, peptidomimetics account for the large majority of FT inhibitors that have been selected for development as candidate antineoplastic agents.

BMS-214662 is a nonpeptidomimetic FT inhibitor composed of a benzodiazepine core with a thienyl sulfonyleurea moiety in the 4-position and a methylimidazole group on the 1-position (Fig. 1; Ref. 3). It is nonsedating; inhibits H-ras and K-ras in vitro with an IC₅₀ of 1.3 and 8.4 nM, respectively; and

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Note: Preliminary results of the study were presented at the 6th Annual Meeting of the American Society for Clinical Oncology (New Orleans, LA), May 2000.

Requests for reprints: David P. Ryan, Massachusetts General Hospital, 55 Fruit Street, Cox Building, Room 640, Boston, MA, 02114. Phone: (617) 724-4000; Fax: (617) 724-3166; E-mail: dpryan@partners.org.
is highly selective for FT over geranylgeranyltransferase I. BMS-214662 has shown potent, selective cytotoxicity against a number of human solid tumor cell lines, including colon, breast, ovarian, prostate, and squamous cell carcinomas. Significant activity against human cancer cell lines transplanted into mice, including HCT-116 (colon), Calu-1 (lung), HT-29 (colon), EJ-1 (bladder), and MiaPaCa-2 (pancreas), has been demonstrated.

In this report, we describe the first clinical trial of BMS-214662 to be performed, in which the drug was given at a single dose i.v. over 1 h every 21 days. The principal objectives of this Phase I clinical trial were to determine the toxicity profile, dose-limiting toxicities (DLTs), and maximum tolerated dose (MTD) for this administration schedule in adult patients with metastatic solid tumors. In addition, studies were performed to characterize the pharmacokinetic behavior of the drug in cancer patients, to evaluate FT inhibition in peripheral blood mononuclear cells (PBMCs), and to describe any evidence of antitumor activity.

Patients and Methods

Patient Selection. The protocol for this clinical trial and the informed consent document were approved by the DanaFarber/Partners Cancer Care (Boston, MA) Scientific Review Committee and Institutional Review Board. All patients were required to provide written informed consent. Eligibility criteria for this trial included the following: histologically confirmed diagnosis of a nonhematological malignancy unresponsive to treatment; measurable or evaluable disease; adequate bone marrow function (absolute neutrophil count ≥ 2000 cells/μl and platelet count ≥ 100,000 cells/μl); adequate hepatic function (total bilirubin ≤ 1.5 mg/dl and alanine aminotransferase/aspartate aminotransferase ≤ 2.5 × the upper limit of normal); adequate renal function (serum creatinine ≤ 1.5 × the upper limit of normal); no more than two prior chemotherapy regimens; at least 18 years of age; Eastern Cooperative Oncology Group performance status score of 0–2; and life expectancy of at least 3 months. Conditions resulting in exclusion from the study included any serious uncontrolled medical disorder or active infection; uncontrolled or clinically significant pulmonary or cardiovascular disease, as indicated by a myocardial infarction within 6 months, atrial or ventricular arrhythmias, congestive heart failure, or QTc ≥ 450 ms; and dementia or altered mental status; females who were either pregnant or lactating were also excluded from the study. Adequate contraception was required for fertile patients.

Treatment Plan. Preliminary evaluations performed within 14 days of starting therapy in the study included a physical examination, medical history, color vision screening, complete blood count, a standard serum chemistry profile, pregnancy test for women of child-bearing potential, electrocardiogram, urinalysis, and tumor measurements by computed tomography scan or other radiographic imaging modality. After initiating treatment, a complete blood count and serum chemistry profile were performed on a weekly basis, with a physical examination and electrocardiogram performed before each course of therapy.

BMS-214662 was supplied by Bristol Myers Squibb Co. (Wallingford, CT) in glass vials containing 250 mg of drug as the free base as a 20 mg/ml solution in sulfobutylether-β-cyclodextrin and citrate buffer. Before administration, it was diluted with 5% Dextrose Injection, USP, to a concentration within the range 0.2–2.5 mg/ml within a polyvinyl chloride i.v. bag. The diluted solution is stable for up to 24 h at room temperature.

The drug was administered as a single i.v. infusion over 1 h through a polyvinyl chloride administration set using a standard volumetric infusion pump. The starting dose, 36 mg/m², represented one-tenth of the dose that produced severe toxicity with a frequency of 10% in rats. The dose was escalated according to a modified Fibonacci scheme. Additional cycles of therapy were repeated every 21 days in patients who continued to satisfy all eligibility requirements. The maximum delay in treatment to permit recovery toxicity from the previous dose was 2 weeks. Therapy was discontinued for any of the following reasons: patient request; noncompliance with protocol; life-threatening toxicity; incomplete recovery from toxicity; progressive disease; or physician decision in light of patient’s other medical conditions. Concomitant use of drugs that are known substrates of cytochrome P450 3A4 was not permitted.

The MTD was established by the occurrence of DLTs during the initial 21-day cycle of therapy. Cohorts of three patients were initially treated at each dose level. Escalation of the dose to the next scheduled level proceeded if there were no DLTs in any of the patients entered into the current dose level during a period of at least 21 days after treatment with the drug. An additional three patients were entered into a dose level if a DLT occurred in one of the initial three patients treated. Dose escalation continued if there were no DLTs in any of these additional patients. The MTD was considered to have been exceeded if at least two patients in a cohort of three to six patients experienced a DLT during the first cycle of therapy, in which case the previous dose was considered the MTD. The MTD would be expanded to accommodate up to 15 patients to determine the suitability of this dose for Phase II studies.

Toxicity Classifications. Toxicities were characterized according to the National Cancer Institute Common Toxicity
Criteria Version 2.0. DLT was defined as any of the following adverse events: (a) grade 4 neutropenia; (b) grade 4 thrombocytopenia; (c) nausea and/or emesis ≥ grade 3 despite the use of maximum medical intervention and/or prophylactic antiemetics; (d) any cardiac, pulmonary, or neurological toxicity ≥ grade 2; (e) any other nonhematological toxicities ≥ grade 3, with the exception of serum hepatic transaminase elevations that resolve to baseline within 2 weeks; and (f) failure to fully recover from toxicity within 35 days after treatment.

Evaluation of Response. A baseline assessment of all measurable disease using any appropriate radiological technique was performed within 21 days before the first cycle of therapy. This included the acquisition of a computed tomography scan for all patients. Evaluations to assess therapeutic response by computed tomography were performed after completing every second cycle of therapy until relapse. Tumor burden was calculated as the sum of the products of the longest perpendicular diameters of all measurable lesions. The duration of a response was measured from the date that the response was first recorded to the date of documented disease progression. Complete response was defined as the disappearance of all measurable disease, signs, symptoms, and biochemical changes related to the tumor. A reduction in tumor burden of ≥50% constituted a partial response. Stable disease was defined as a <50% decrease in tumor burden or an increase than did not exceed 25%. In addition, for each of these classifications, the response or disease stabilization had to persist for a minimum of 4 weeks during which time no new lesions were detected. Progressive disease was indicated by a ≥25% increase in tumor burden or the appearance of any new lesion.

Clinical Pharmacology Studies. Sampling to define the plasma pharmacokinetics of BMS-214662 was performed during the first and second cycles of therapy. Blood samples (7 ml) were drawn from a peripheral vein directly into tubes with freeze-dried sodium heparin before dosing and at 30, 60, 75, 90, and 105 min and 2, 3, 4, 6, 8, and 24 h after starting the 1-h infusion of drug. Sample tubes were mixed by inversion and placed on wet ice until centrifuged (1000 × g, 10 min, 4°C) within 15 min. The plasma was removed and stored at −70°C until assayed.

The concentration of BMS-214662 in plasma was determined by an analytical method involving reversed-phase high performance liquid chromatography with UV detection. After allowing frozen plasma samples to thaw at room temperature, a 500-μl aliquot of plasma was mixed with 0.5 ml of ammonium phosphate buffer [1 M (pH 7.0)] and extracted with 5 ml of 1-chlorobutane. After centrifuging for 10 min (5000 rpm), the organic phase was removed, and the solvent was evaporated under a stream of nitrogen at 30°C. The residue was reconstituted in 300 μl of acetonitrile-ammonium acetate buffer [50 mM (pH 4.7); 30:70 (v/v)], and a 100-μl aliquot was loaded onto a Luna C18 –2 (4.6 mm inner diameter × 150 mm, 5-μm particle size) analytical column (Phenomenex, Torrance, CA). The separation was performed at 30°C using a binary mobile phase composed of ammonium acetate buffer [50 mM (pH 4.7)] and acetonitrile, delivered at a flow rate of 1.0 ml/min. The amount of acetonitrile in the mobile phase was maintained at 50% for the initial 2.5 min of the run and then linearly increased to 100% from 2.5 to 7.0 min. The column was flushed with 100% acetonitrile for an additional 1.5 min before decreasing back to 50% over 1.5 min and allowing the system to re-equilibrate for 5 min before the next injection. The drug eluted with a retention time of 5.2 min and was monitored by UV absorbance at 305 nm.

Study samples were assayed together with a series of nine calibration standards of BMS-214662 in plasma at concentrations ranging from 4.3 to 4000 ng/ml in terms of the free base form of the drug. The relationship between the chromatographic peak height and known drug concentration for each calibration standard was analyzed by linear regression using the reciprocal of the nominal concentration of the standards as the weighting factor. Values of the parameters describing the best-fit line were used to calculate the BMS-214662 concentration in study samples. The accuracy of the assay ranged from 0.8 to 6.5% with a precision of 3.2–5.2% for the analysis of quality control samples with nominal concentrations of 15, 749, and 3200 ng/ml. At the lower limit of quantitation, 4.3 ng/ml, values of the accuracy and precision were 6.1% and 14.4%, respectively.

PBMCs were isolated from blood specimens obtained before dosing, at the end of the 1-h infusion of drug, and at 6 and 24 h after starting the infusion for assessing FT activity. Additional samples were obtained at 1 and 3 h postinfusion from the expanded cohort of patients treated at the MTD. Blood samples (7 ml) were collected in Vacutainer CPT mononuclear cell preparation tubes containing sodium citrate buffer (Becton Dickinson), mixed by inversion, and maintained at ambient temperature until centrifuged (1700 × g, 30 min, 25°C) in a swinging bucket rotor within 60 min. After aspirating the plasma, the lymphocyte/monocyte band was carefully removed and transferred into a 15-ml polypropylene centrifuge tube. The cells were washed twice with 10 ml of ice-cold PBS. The cell pellet afforded by centrifugation at 300 × g for 15 min at 4°C was stored directly at −70°C until assayed after completely removing the buffer.

FT activity was determined by a radioenzyme assay in which [3H]farnesylated ras is produced from the addition of [3H]farnesylpyrophosphate and synthetic human H-ras to endogenous FT present in PBMC lysates. Radioactivity associated with [3H]farnesylated ras captured on a microplate filter was measured by liquid scintillation counting. Lysis buffer containing 20 mM HEPES and sodium chloride, 1× Complete Protease Inhibitor Mixture (Boehringer Mannheim Diagnostics, Indianapolis, IN), 0.01% NP40 detergent (Boehringer Mannheim), and 1 mM DTT was prepared on a daily basis. PBMC pellets were thawed on ice, gently mixed with 50 μl of lysis buffer, and centrifuged at 11,000–14,000 rpm for 10 min at 4°C. The concentration of protein in the cell-free lysates was determined on a microtiter plate using the Detergent Compatible Protein Assay Kit (Bio-Rad, Hercules, CA). Lysates were adjusted to a protein concentration of approximately 2 mg/ml (10 μg/sample) with lysis buffer. H-ras proteins were expressed in bacteria and purified as described previously (4). A 10× buffer solution containing 1 m HEPES, 0.25 m magnesium chloride, 0.1 mM zinc chloride, and 0.1 mM DTT was prepared daily and kept on ice until used. A mixture of H-ras proteins, 10× buffer, deionized water, and 22 μM [3H]farnesylpyrophosphate (New England Nuclear, Boston, MA) was prepared and kept on ice until used.

Each PBMC lysate was assayed in triplicate together with
controls consisting of blank lysis buffer and purified recombinant human FT on a 96-well microtiter plate (3). The ras protein mixture (15 μl) was pipetted into each well of a microtiter plate, to which 5 μl of PBMC lysate or control solution were added, and mixed with the pipetter. The plate was sealed, briefly vortexed, and incubated at 37°C for 1 h. The reactions were terminated by adding 90 μl of 4% SDS, after which 90 μl of 30% trichloroacetic acid were pipetted into each well and thoroughly mixed to precipitate protein. After refrigeration of the plate for 90 min, the contents of each well were quantitatively transferred to a corresponding 96-well multiscreen filtration plate, and vacuum was applied to collect the [3H]farnesylated H-ras onto filter membranes (Millipore Corp., Milford, MA). Each well of the original microtiter plate was rinsed with a solution of 4% SDS in 6% trichloroacetic acid, and the rinse was transferred to and filtered through the multiscreen plate. The wells of the filtration plate were rinsed five times with 6% trichloroacetic acid to assure adequate protein binding to the filters. After the liquid was completely filtered through the membranes, the plate was removed from the vacuum manifold, and the plate bottom was blotted on paper towels. After air drying for 10 min, the filter plate was placed into a Millipore filter punch apparatus, and the filter membranes were punched from each well of the plate directly into 7-ml scintillation vials containing 300 μl of Solvable protein solubilizing reagent (Packard Co., Downers Grove, IL). The membranes were digested at 60°C for 20 min. After cooling to room temperature, 3 ml of Formula 989 liquid scintillation mixture (Packard Co.) were added to each vial. The capped vials were vigorously mixed on a multitube vortex mixer for 15 s. Radioactivity was measured with a Packard TR2500 scintillation counter. FT activity in each sample was determined as fmol/μg protein/h and reported as a percentage of the activity in the pretreatment sample for each patient. The arithmetic mean of the normalized FT activity was calculated from the determinations made at each time point for the group of patients in each dose level.

Actual sample times were calculated from the beginning of the drug infusion to the midpoint of each sample collection interval. Individual patient plasma concentration-time data were analyzed by noncompartmental methods using routines supplied in the WinNonlin Version 1.1 software package (Scientific Consulting, Apex, NC). The area under the plasma concentration-time profile from time 0 to infinity (AUC) was estimated by the logarithmic-linear trapezoidal algorithm to the data point at approximately 8 h, with extrapolation to time infinity using the slope of the terminal log concentration versus time data. Total plasma clearance (CL) was calculated as the dose divided by the AUC. Mean values of the pharmacokinetic variables were calculated as the geometric mean of the individual patient values (5, 6). SDs for the geometric mean values were estimated by the jackknife method (7). Pearson correlation coefficients (r) were calculated to identify relationships between the AUC of BMS-214662 and continuous demographic characteristics. Parametric statistical tests of pharmacokinetic variables were performed after logarithmic transformation of the data. All tests were two-sided, and values of |r| ≥ 0.4 and/or P < 0.05 was used as the criteria for significance.

**Results**

**Patient Characteristics.** Characteristics of the 44 patients evaluated during the course of this study are listed in Table 1. The median age of the study population was 54 years (range, 23–74 years), and the study population was composed of 22 women and 22 men. The majority of patients (64%) had an Eastern Cooperative Oncology Group performance status of 1. Pancreatic cancer (n = 14), lung cancer (n = 7), and colorectal cancer (n = 5) were the most common tumor types. The patients were heavily pretreated: 69% of the cohort had received at least two prior regimens of chemotherapy; and 22 (50%) of the patients had prior radiotherapy.

**DLTs and MTD.** The dose was escalated from 36 to 225 mg/m² through five intermediate dose levels (Table 2). DLTs that occurred during the first cycle of therapy included reversible transaminitis, diarrhea, nausea, and vomiting. An additional three patients were enrolled for evaluation at the starting dose when one of the first three patients developed reversible grade 3 transaminitis. A fourth patient was added to the cohort at dose level 2 (60 mg/m²) when a patient with pancreatic cancer experienced grade 3 hyperbilirubinemia due to biliary obstruction from the tumor and was consequently deemed inevaluable. At the fourth dose level (126 mg/m²), an additional three patients were added when a patient experienced grade 3 hyperbilirubinemia and grade 3 transaminitis, again due to biliary obstruction by tumor. Among the first six patients enrolled at 225 mg/m², the highest dose administered, two patients had reversible grade 3 transaminitis.

Because the transaminitis was completely reversible and did not appear to have any clinical adverse effect, the protocol was amended to exclude reversible grade 3 transaminitis as a DLT and to explore an intermediate dose level of 200 mg/m². The initial three patients that received this dose tolerated the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Patient characteristics</th>
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<tbody>
<tr>
<td>Characteristic</td>
<td>No. of patients</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>Median 54, Range 23–74</td>
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<td>Prior chemotherapy regimens</td>
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*ECOG, Eastern Cooperative Oncology Group.*
Phase I Trial of BMS-214662

Table 2  Dose levels and treatment data

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. of patients</th>
<th>No. of courses of therapy delivered</th>
<th>Range within patients</th>
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<tr>
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<td>1–4</td>
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<td>1–6</td>
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<td>90</td>
<td>3</td>
<td>6</td>
<td>1–2</td>
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<tr>
<td>126</td>
<td>6</td>
<td>36</td>
<td>1–18*</td>
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<tr>
<td>168</td>
<td>3</td>
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<td>1–6</td>
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<td>200</td>
<td>9</td>
<td>23</td>
<td>1–5</td>
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<tr>
<td>225</td>
<td>13</td>
<td>23</td>
<td>1–5</td>
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Table 3  Summary of clinical toxicities

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<tr>
<th>Dose level (mg/m²)</th>
<th>Nausea</th>
<th>Vomiting</th>
<th>Diarrhea</th>
<th>Elevated AST</th>
<th>Elevated ALT</th>
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<td>1/1</td>
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</table>

* AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Drug without any severe toxicities. Accordingly, the dose was increased back to 225 mg/m², where seven additional patients were ultimately enrolled. Among the 13 patients treated with 225 mg/m² BMS-214662, DLTs occurred in 3 patients (grade 3 nausea/vomiting in 2 patients and grade 3 diarrhea in 1 patient). In addition, four patients had reversible grade 3 transaminitis, and seven patients experienced ≥grade 2 nausea/vomiting despite treatment with serotonin receptor antagonists. Although the DLT rate by definition of the protocol did not meet criteria for declaring the 225 mg/m² dose the MTD, the degree of nausea and vomiting was intolerable and inappropriate for Phase II studies. Therefore, an additional six patients were enrolled for treatment with a dose of 200 mg/m². Among the entire cohort of nine patients evaluated at this dose level, one patient had grade 3 diarrhea and another had grade 3 nausea/vomiting. In addition to these two DLTs, reversible grade 3 transaminitis occurred in three patients. Although the MTD as written by the protocol had not been reached, the overall tolerability of this dose was considered to be acceptable and appropriate for Phase II studies using this administration schedule of BMS-214662.

Toxicities. There were no drug-related adverse events greater than grade 3 in severity. Hematological toxicity was very mild, and only one patient treated with a dose of 200 mg/m² experienced grade 3 neutropenia. There were no other episodes of grade 2 or more severe neutropenia or thrombocytopenia. Among the nonhematological toxicities (Table 3), nausea and transaminitis were the most common side effects, with each occurring in 8 of 13 (62%) patients treated with the 225 mg/m² dose of the drug. Fatigue that appeared to be possibly related therapy was experienced by 41% of the patients. Dizziness or lightheadedness that could have been drug related occurred in 14% of the patients, and one patient had grade 2 ataxia after receiving benzodiazepine for treatment of nausea. Three patients reported seeing flashing lights, and two patients reported blurred or double vision during therapy that was considered to be treatment related and transient.

Antitumor Activity. There were no objective responses noted; however, there were suggestions of therapeutic benefit to treatment with the drug. One patient with locally advanced pancreatic cancer has prolonged stable disease and has been receiving 126 mg/m² BMS-214662 for more than 3.5 years. This patient had progressive disease while receiving gemcitabine before enrollment into this study. One patient with metastatic small-cell lung cancer had a 40% decrease in the size of a liver metastasis and complete disappearance of one of three small brain metastases. This patient remained on the study for 17 months and then was removed for progressive disease. A patient with colon cancer had diminished ascites for 15 weeks before progressing.

Pharmacokinetics. The plasma pharmacokinetics of BMS-214662 was defined during treatment with the first dose of drug in 40 of the 44 patients. Pharmacokinetic samples were not obtained from one patient treated at the 200 mg/m² dose level, and the data were not amenable to analysis for an additional three patients due to problems encountered with venous access that precluded the collection of a sufficient number of samples. Geometric mean plasma concentration-time profiles of BMS-214662 determined in patients treated at four dose levels ranging from 36 to 225 mg/m² are shown in Fig. 2. Plasma levels of the drug increased very rapidly after initiating the infusion of drug, as indicated by the close similarity between the concentrations observed at the midpoint and end of the 60-min infusion. The maximum plasma concentration (C_max) increased from a mean value of 1.03 ± 0.21 μg/ml in the group of 6 patients treated with the starting dose of 36 mg/m² to 5.77 ± 2.38 μg/ml in the cohort of 12 patients evaluated at the 225 mg/m² dose level.
Table 4 Mean values of BMS-214662 pharmacokinetic parameters determined during the first cycle of therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose level (mg/m²)</th>
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<tr>
<td></td>
<td>36</td>
</tr>
<tr>
<td>No. of patients</td>
<td>6</td>
</tr>
<tr>
<td>$C_{max}^a$ (μg/ml)</td>
<td>1.03 ± 0.21$^b$</td>
</tr>
<tr>
<td>$t_{1/2,\alpha}$ (h)</td>
<td>2.02 ± 0.42</td>
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<tr>
<td>MRT (h)</td>
<td>1.08 ± 0.27</td>
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<tr>
<td>AUC (μg h/ml)</td>
<td>1.24 ± 0.22</td>
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<tr>
<td>CL (liters/h/m²)</td>
<td>29.0 ± 5.2</td>
</tr>
<tr>
<td>$V_{ss}$ (liters/m²)</td>
<td>31.2 ± 10.6</td>
</tr>
</tbody>
</table>

$^a$ $C_{max}$, maximum plasma concentration; $t_{1/2,\alpha}$, apparent biological half-life; MRT, mean residence time; AUC, area under the plasma concentration-time profile from time 0 to infinity; CL, total plasma clearance; $V_{ss}$, apparent volume of distribution at steady state.

$^b$ Geometric mean ± SD.

Sampling to define the plasma profile of BMS-214662 was also performed during the second cycle of therapy in 13 of the patients treated at the 200 and 225 mg/m² dose levels to assess intrapatient variability in the pharmacokinetics of the drug. Grand mean values of the pharmacokinetic parameters determined during the first and second cycles of therapy are listed in Table 5. There were no significant differences in any of the pharmacokinetic parameters between the first and second cycles of therapy.

Body surface area values of the patients ranged from 1.37 to 2.29 m² (median, 1.88 m²). The mean ± SD CL of BMS-214662 in cycle 1 was 42.6 ± 20.9 liters/h when calculated without normalizing to body surface area. Interpatient variability in the CL was not markedly improved when normalized to body surface area (CV, 42.6% versus 45.6%). The relationship between the unnormalized CL values (liters/h) and body surface area yielded a Pearson correlation coefficient of 0.396, suggestive of a moderate but significant association ($P = 0.012$). There were no statistically significant differences in the mean CL of the drug in patients grouped according to gender, age (<50 years versus >50 years), or performance status.

**Pharmacodynamics.** FT activity in PBMCs (expressed as a percentage of the pretreatment baseline activity) determined after administering the first dose of BMS-214662 is presented in Table 6. Baseline activity determinations for five patients were not acceptable, and samples were not obtained from another two patients. In most patients, maximum inhibition of the enzyme occurred in the sample obtained near the end of the 1-h infusion. FT activity recovered gradually during the initial 5 h after completing the infusion and approached baseline values within 24 h after dosing. The average FT activity decreased from

![Fig. 3 Plots demonstrating the relationship between the maximum plasma concentration ($C_{max}$; A) and area under the plasma concentration-time profile from time zero to infinity (AUC; B) values of BMS-214662 and the initial dose. Points (O) represent the observed values in individual patients, and the horizontal bars depict the geometric mean values obtained for each dose level. The solid line was generated from linear regression analysis of the geometric mean values at each dose.](image-url)

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63.1 ± 14.0% to 10.1 ± 8.1% at the end of the infusion as the dose of BMS-214662 was increased from 36 to 126 mg/m². Further escalation of the dose by a factor of almost 2-fold did not result in greater inhibition of the enzyme in PBMCs.

The relationship between FT activity in PBMCs at the end of the infusion and the Cₘₕₜ of BMS-214662 is depicted in Fig. 4, together with the curve generated by fitting the inhibitory sigmoid maximum effect model

\[ E = E_{\text{max}} \cdot \left( \frac{1 - C_{\text{max}}}{C_{\text{max}} + C_{50}} \right) \]

to the experimental data by weighted (1/yᵦₑᵣᵣ) nonlinear regression (8). In this equation, the pharmacological effect E is the corresponding FT activity expressed as a percentage of baseline, \( E_{\text{max}} \) is the effect when the \( C_{\text{max}} \) is equal to 0, \( C_{50} \) is the \( C_{\text{max}} \) corresponding to 50% of the maximum effect, and \( \lambda \) is the sigmoidicity parameter. \( E_{\text{max}} \) was fixed to a value of 100 because the FT activity data were normalized to the baseline value in each patient. The \( C_{\text{max}} \)-effect relationship exhibited a steeply decreasing region that was approximately log-linear. In general, there was good agreement between the best-fit equation and experimental data. Estimated values of \( C_{50} \) and \( \lambda \) were 1.2 μg/ml and 1.6, respectively, with coefficients of variation of <25%.

**Discussion**

BMS-214662 is a low molecular weight benzodiazepine derivative that selectively inhibits FT and has shown excellent activity against human tumor xenograft models. It is unique among farnesyltransferase inhibitors that have been selected for development as candidate chemotherapeutic agents in that it is a nonpeptidomimetic compound and has potent cytotoxic activity against solid tumor cell lines. In this initial clinical trial, we evaluated the administration of BMS-214662 to patients with advanced solid tumors as a single 1-h i.v. infusion repeated every 21 days. Early in the study, it became apparent that BMS-214662 caused reversible transaminitis, prompting an amendment to the study protocol to exclude reversible grade 3 transaminitis as a DLT. The principal toxicities of the drug that prevented further dose escalation were nausea, vomiting, and diarrhea. Despite DLTs occurring in only 3 of 13 patients (23%), the MTD was declared at 225 mg/m² due to an unacceptable level of at least grade 2 nausea and vomiting, thereby establishing 200 mg/m² as the dose recommended for Phase II studies of this regimen.

The drug showed evidence of clinical activity in three patients: resolution of ascites in a patient with colon cancer; a 40% reduction in liver and brain metastases in a patient with non-small cell lung cancer; and stable disease lasting for >40 months in a patient with pancreatic cancer. The latter patient was diagnosed with locally advanced disease at the time of an exploratory laparotomy and had biopsy-proven adenocarcinoma. Although this an interesting observation, whether this particular patient’s long-term survival is directly attributable to treatment with BMS-214662 is unclear.

BMS-214662 exhibits linear pharmacokinetics in cancer patients and is eliminated rather rapidly from systemic circulation. The \( t_{1/2} \) of the compound was only 90 min on average, and its \( V_{\text{ss}} \) was also very low. Preclinical studies revealed that BMS-214662 is a substrate and inhibitor of cytochrome P450 isoform 3A4 [CYP3A4 (3)]. Accordingly, the use of any other drugs that are known to significantly induce or inhibit hepatic CYP3A4 within 7 days of initiating therapy with BMS-214662.

**Table 6** Inhibition of FT* activity in PBMCs after treatment with BMS-214662

<table>
<thead>
<tr>
<th>Dose (mg/m²)</th>
<th>No. of patients</th>
<th>FT activity (% of baseline) at sample times of</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>36</td>
<td>4</td>
<td>63.1 ± 14.0b</td>
<td>c</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
<td>42.1 ± 2.0</td>
<td>c</td>
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<td>c</td>
</tr>
<tr>
<td>126</td>
<td>5</td>
<td>10.1 ± 8.1</td>
<td>c</td>
</tr>
<tr>
<td>168</td>
<td>2</td>
<td>25.0 ± 19.0</td>
<td>c</td>
</tr>
<tr>
<td>200</td>
<td>8</td>
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</tr>
<tr>
<td>225</td>
<td>12</td>
<td>15.2 ± 14.2</td>
<td>20.5 ± 20.0</td>
</tr>
</tbody>
</table>

*b* FT, farnesyltransferase; *PBMC*, peripheral blood mononuclear cell.

**Fig. 4** Relationship between the farnesyltransferase activity at the end of the 1-h infusion of the drug and the maximum plasma concentration (Cₘₜₚ) of BMS-214662. The solid curve was generated by fitting the experimental data to an inhibitory sigmoidal maximum pharmacologic effect (Eₘₜₚ) equation.
was not permitted to avoid any potential for a pharmacokinetic drug interaction. Nevertheless, four subjects had received several medications that could potentially modulate CYP3A4 activity. Two patients who continued to receive fluoxetine, a known inhibitor CYP3A4, upon entering the study had a substantially lower CL of BMS-214662 (6.0 and 13.8 liters/h/m²) than the mean value for the entire cohort (21.8 ± 10.8 liters/h/m² (9)). In addition, drug elimination appeared to be enhanced in two patients who concurrently received drugs that are known inducers of hepatic CYP3A4. One patient who continued to receive a long-term anticonvulsant regimen consisting of phenytoin and phenobarbital had a CL of 38.9 liters/h/m². A greater than average CL of 40.3 liters/h/m² was also evident in a female patient who was being treated with a stable dose of oral progesterone. Thus, there is some evidence consistent with pharmacokinetic drug interactions resulting from both the induction and inhibition of the hepatic metabolism of BMS-214662. Caution in the administration of BMS-214662 together with other medications that are known modulators of hepatic drug metabolizing enzymes is therefore indicated.

The extent and duration of FT inhibition in PBMC were closely associated with the plasma concentration of BMS-214662 in a time-dependent manner. In patients treated with the recommended Phase II dose of 200 mg/m², FT activity was not completely inhibited, and the duration of inhibition was relatively transient. The maximum level of inhibition was 10% of the pretreatment activity occurring near the end of the infusion, recovering to 50% of baseline within 7 h after dosing and completely reversed by 24 h. The Cmax of BMS-214662 achieved with the recommended dose for Phase II studies (200 mg/m²), 13.4 ± 4.9 µM, was substantially greater than the IC50 for inhibiting the farnesylation of H-ras (1.3 nM) and K-ras (8.4 nM) in vitro. However, continuous exposure to a concentration of 300 nM for 72 h was required to convert H-ras-transformed Rat1 cells to a normal flat layer phenotype. The induction of apoptosis in cancer cells in vitro required continuous exposure to 370 nM of the drug for 24 h. In contrast, plasma levels of the drug that inhibit cancer cell growth in vitro decayed to a mean value of 201 nM in patients 7 h after administration of a 200 mg/m² dose as a 1-h i.v. infusion. More sustained and complete inhibition of the enzyme may be required if FT inhibition is indeed essential to interrupt ras signaling of mitogen-activated protein kinase and AKT, thus inducing apoptosis (10–13). Administration of the drug as a continuous i.v. infusion could potentially facilitate greater and more prolonged FT inhibition than that achieved after administration as a 1-h i.v. infusion.

FT inhibitors have shown only marginal activity in patients with solid tumors in previous clinical trials (14–20). However, several of these compounds, including BMS-214662, have demonstrated activity in patients with leukemia and myelodysplastic syndrome (21–23). The limited clinical efficacy of FT inhibitors against solid tumors raises the questions of whether these agents are effectively inhibiting ras and whether inhibition of ras is a useful target in solid tumors. Given the large number of farnesylated proteins in cells, it is possible that FT inhibitors exert their antitumor effect through other pathways such as phosphatidylinositol 3’-kinase or Rho-B (10, 11, 13, 24, 25).

In conclusion, BMS-214662 can be delivered safely as a 1-h i.v. infusion every 21 days to patients at doses that achieve plasma levels of the drug that inhibit cancer cell growth in vitro. Reversible grade 3 transaminitis, nausea, and vomiting were the most frequent adverse effects of the drug. Hints of antitumor activity were seen, particularly in a patient with pancreatic cancer who continues to receive therapy after 3.5 years, but no patient experienced an objective response. Because FT inhibition in PBMCs was short-lived with the 1-h infusion schedule, further evaluation of this compound is warranted with schedules that may provide more prolonged systemic exposure to the drug.

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Phase I Clinical Trial of the Farnesyltransferase Inhibitor BMS-214662 Given as a 1-Hour Intravenous Infusion in Patients with Advanced Solid Tumors


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