Cancer Chemother Pharmacol (2006) 58: 107–116
DOI 10.1007/s00280-005-0134-0

ORIGINAL ARTICLE

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Phase I clinical trial of the farnesyltransferase inhibitor BMS-214662 administered as a weekly 24 h continuous intravenous infusion in patients with advanced solid tumors

Received: 17 March 2005 / Accepted: 27 September 2005 / Published online: 13 December 2005

Abstract *Purpose*: BMS-214662 is a novel farnesyltransferase (FT) inhibitor that has shown promising suggestions of single agent activity in patients with advanced solid tumors when administered as a 1 h intravenous (i.v.) infusion every 3 weeks. The degree of FT inhibition in peripheral blood mononuclear cells (PBMCs) was greatest at the end of the infusion and rapidly reversed as the concentration of the drug in the plasma decayed. A second phase I trial of BMS-214662 administered as a weekly 24 h i.v. infusion was initiated to determine if the duration of maximum FT inhibition could be significantly extended by prolonging the infusion time and increasing the frequency of administration. *Patients and methods*: Infusion of BMS-214662 was prolonged from 2, 4, 8, 16, 24 h in single patient cohorts and repeated weekly for 3 out of 4 weeks. The initial dose was 56 mg/m^2. When the infusion duration reached 24 h, the dose was escalated at a constant multiples of 1.4 in single patient cohorts until the occurrence of toxicity greater than grade 1, upon which groups of at least three patients were evaluated at each dose level. The plasma pharmacokinetics and FT inhibition in PBMCs were measured in all patients at the prospective maximum tolerated dose. *Results*: Nineteen patients participated in the study (11 males/8 females) and the weekly dose was increased to a maximum of 300 mg/m^2 given as a 24 h i.v. infusion. Drug-related toxicity greater than grade 1 first occurred at 300 mg/m^2, with two patients experiencing dose-limiting toxicity. One patient developed a grade 3 hyponatremia and another developed reversible grade 3 diarrhea, grade 2 renal toxicity, and grade 3 transaminitis. A 275 mg/m^2 dose was then evaluated, where one of the three patients treated experienced reversible grade 4 renal toxicity and grade 3 diarrhea. In view of the identical renal toxicity at 275 mg/m^2 in another study and limited drug availability, there was no further accrual to this dose level and the study was closed. No evidence of antitumor activity was observed. The plasma pharmacokinetics of BMS-214662 was linear with high interpatient variability. In the three patients evaluated at the 275 mg/m^2 dose level, the maximum inhibition of FT activity in PBMCs was $47 \pm 23\%$ of the baseline. *Conclusion*: Administering BMS-214662 as a weekly 24 h continuous i.v. infusion permitted a considerably greater dose intensity to be delivered as compared to a single 1 h infusion given once every 3 weeks. The more prolonged infusion schedule resulted in a much lower degree of maximum FT inhibition in PBMCs than achieved with the 1 h infusion, although the duration of enzyme inhibition was longer, consistent with the lower peak plasma concentration of the drug provided by comparably tolerated doses when given as a 24 h infusion. Similarly, delivering the drug with increased dose intensity permitted by this weekly administration schedule did not appear to enhance its

Preliminary results were presented at the 38th annual meeting of the American Society for Clinical Oncology, May, 2002, Orlando, FL, USA.

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therapeutic benefit, at least in this phase I trial. Continued development of BMS-214662 may depend upon the potential for using it in combination with other anticancer drugs.

Keywords Cancer · Chemotherapy · Human · Pharmacokinetics · Pharmacodynamics

Introduction

The *ras* oncogene family represents a particularly attractive target for anticancer therapy because activating mutations of the *ras* genes are among the more common genetic aberrations known in human cancers [1]. The mutations found in cancer cells produce a constitutively active protein that does not require exogenous stimulation. The proteins encoded by the *ras* genes are guanine nucleotide binding proteins, which associate with the inner plasma membrane and transduce external signals to the interior of the cell [2]. They are among the limited set of known proteins that undergo post-translational prenylation, in this case farnesylation, a reaction catalyzed by the cytosolic enzyme farnesyltransferase (FT) [3]. This modification allows ras proteins to anchor to the inner surface of the plasma membrane and is required for normal functions in signal transduction as well as for transforming activities. Consequently, inhibiting farnesylation severely impairs the function of ras proteins [1–3].

BMS-214662 is a tetrahydrobenzodiazepine derivative that selectively inhibits FT and has shown excellent activity against human tumor xenograft models [4]. Promising evidence of clinical activity was observed in three patients during the initial phase I clinical trial of BMS-214662 given as a single 1 h intravenous (i.v.) infusion repeated every 3 weeks [5]. Ascites resolved in a patient with colon cancer, a patient with non-small cell lung cancer showed a greater than 40% reduction in liver and brain metastases, and prolonged disease stabilization (>48 months) occurred in a patient with pancreatic cancer. There was a close association between inhibition of FT activity in peripheral blood mononuclear cells (PBMCs) and the concentration of BMS-214662 in plasma. In patients treated with the dose recommended for phase II studies, 200 mg/m^2, the maximum decrease in enzyme activity to 10.5% of the baseline occurred at the end of the 1 h infusion. Thereafter, FT activity recovered in parallel with the relatively rapid decline of the drug concentration in plasma, becoming completely reversed by 24 h after dosing.

Based upon these findings, a second phase I study of BMS-214662 was undertaken to determine if prolonging the infusion time and increasing the frequency of administration could significantly extend the duration of maximum FT inhibition. The drug was administered as a 24 h continuous i.v. infusion every 7 days for three consecutive weeks during each 4 week cycle to adult patients with unresponsive, advanced solid tumors. An accelerated dose escalation method was used to establish the maximum tolerated dose (MTD) for this administration schedule [6]. FT inhibition in PBMCs was performed to relate this to the plasma pharmacokinetics of the drug.

Patients and methods

Patient selection

The protocol for this clinical trial and the informed consent document were approved by the Dana-Farber/Partners Cancer Care (Boston, MA, USA) Scientific Review Committee and Institutional Review Board. All patients were required to provide written informed consent. Eligibility criteria were identical to our previous clinical trial of BMS-214662 given as a 1 h i.v. infusion [5]. Briefly, patients were required to be at least 18 years old and have a histologically documented refractory solid tumor, an Eastern Cooperative Oncology Group performance status of 0–2, adequate hematological (absolute neutrophil count >1,500/μl, platelets >10 e5/μl), hepatic (ALT, AST and alkaline phosphatase <2.5 times the upper limits of normal, normal bilirubin) and renal (creatinine < upper limits of normal) function, with no other clinically significant medical disorders. Preliminary evaluations performed within 14 days of beginning treatment in the study included a medical history, physical examination and performance status determination, an electrocardiogram and chest X-ray, a complete blood count with platelet and differential counts, coagulation tests (prothrombin time, partial thromboplastin time), a standard serum chemistry profile, urinalysis, pregnancy test for women of child-bearing potential, and radiographic tumor measurement.

Drug administration

BMS-214662 was supplied by the Bristol-Myers Squibb Company (Wallingford, CT, USA) in glass vials containing 250 mg of drug (free base) as a 20 mg/ml solution in sulfobutylether-β-cyclodextrin and citrate buffer. This was diluted with 5% Dextrose injection, USP to a concentration of 0.2–2.5 mg/ml in a polyvinyl chloride infusion bag for administration. The starting dose of 56 mg/m^2 was one-third of the lowest non-toxic dose of BMS-214662 in cancer patients when given as a single 1 h i.v. infusion [5]. The drug was administered once every 7 days for three consecutive weeks. Additional cycles of therapy were repeated every 28 days in patients who continued to satisfy all eligibility requirements. In the initial portion of the study, the duration of infusion was incrementally extended from 2 to 4, 8, 16 and 24 h in single patient cohorts, whereupon dose escalation

proceeded using the 24 h i.v. infusion. The dose was escalated by a constant factor of 1.4 relative to the preceding dose level in single patients, until the first occurrence of a drug-related toxicity greater than grade 1, after which the dose was increased by a factor of 1.3 in groups of at least three patients to establish the MTD. The MTD was based upon the dose-limiting toxicity (DLT) that occurred during the initial 28 day cycle of therapy. Dose escalation proceeded in the absence of a DLT in any patient(s) evaluated at a given dose level. An additional three patients were introduced 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 exceeded if at least two patients in a cohort of 3–6 experienced a DLT, thereby defining the previous dose as the MTD.

Toxicity assessments

As patients were actively enrolled in the study a physical examination, electrocardiogram, urinalysis and determination of hematologic and serum chemistry parameters were performed on a weekly basis and also one month after administering the last dose of drug to patients removed from the study. Toxicities were characterized according to the current version of the National Cancer Institute Common Toxicity Criteria version 2.0 (http://ctep.cancer.gov/forms/ctcv2nom-4-30-99-final3.pdf). DLT was defined as any of the following adverse events occurring duration cycle 1 only: (a) grade 4 neutropenia of any duration; (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 neurologic toxicity ≥ grade 2; (e) any other non-hematological toxicities ≥ grade 3, with the exception of serum hepatic transaminase elevations that resolve to the baseline within 2 weeks; (f) failure to fully recover from toxicity within 35 days after beginning treatment.

Evaluation of response

Tumor measurements were performed and the response to therapy was defined as previously described by modified WHO criteria [5]. A baseline assessment of all measurable disease using any appropriate radiological technique was performed within 21 days prior to the first cycle of therapy. This included the acquisition of a computed tomography (CT) scan for all patients. Evaluations to assess therapeutic response by CT 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% or greater constituted a partial response. Stable disease was defined as a less than 50% decrease in tumor burden or an increase that 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 greater than 25% increase in tumor burden or the appearance of any new lesion.

Pharmacokinetic and pharmacodynamic studies

Sampling to determine FT activity in PBMCs and the plasma pharmacokinetics of the drug was performed during treatment with the first dose of cycle 1. FT activity was measured in patients treated with the MTD, as previously reported [5], in cytosolic extracts of PBMCs isolated from blood specimens obtained before dosing, at the midpoint and end of infusion, and 24 and 48 h postinfusion. Pharmacokinetic blood samples (7 ml) were drawn from a peripheral vein directly into tubes with freeze-dried sodium heparin. For patients receiving the drug as a 24 h infusion, samples were collected shortly before dosing, 0.5, 1, 2, 4, 22 and 23 h after starting the infusion, 5 min before the end of the infusion, and then at 10 min and 0.5, 1, 2, 4, 6, 22 and 24 h after the end of the infusion. Some adjustment of the sample collection times was necessary for the shorter duration infusions. Sample tubes were mixed by inversion and placed on wet ice until centrifuged (1,000 *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 high performance liquid chromatography (HPLC) with electrospray ionization mass spectrometric (EI-MS) detection. An analytical reference sample of the drug, as the methansulfonate salt, was provided by Bristol-Meyers Squibb (Princeton, NJ, USA). Frozen study samples were permitted to thaw at ambient temperature and were briefly vortexed. Samples (50 μl) were prepared for analysis by vigorously mixing with 100 μl of acetonitrile containing the internal standard, 1-α-naphthoflavone (Sigma, St. Louis, MO, USA) at a concentration of 6.0 ng/ml. The mixture was centrifuged for 5 min at 10,000 *g* to pellet the precipitated plasma proteins upon which 100 μl of the clear supernatant was diluted with 100 μl of 25 mM ammonium formate solution. The prepared sample solution (100 μl) was loaded onto a 15 cm×3.9 mm I.D. NovaPak phenyl (4 μm particle size) HPLC column (Waters Corp., Milford, MA, USA), preceded by a 15 mm × 3.2 mm I.D. Brownlee New Guard phenyl precolumn (Alltech, Deerfield, IL, USA) and a 0.5 μm inline filter (Upchurch Scientific, Oak Harbor, WA, USA). An isocratic mobile phase composed of methanol–10 mM ammonium for-

mate (70:30, v/v) delivered at 1.0 ml/min was used for the separation at ambient temperature. EI-MS detection was performed using an Agilent Technologies (Palo Alto, CA, USA) 1100 Series LC/MSD system. Operating parameters of the atmospheric pressure ionization-electrospray interface were: nebulizer pressure, 40 p.s.i.; drying-gas, N_2; drying-gas flow, 12 l/min; drying-gas temperature, 350°C; capillary potential, 2,000 V. The single-quadrupole mass spectrometer was operated in the selected-ion monitoring mode to measure positive ions corresponding to the protonated molecule for BMS-214662 at m/z 490.1 and at m/z 273.0 for the internal standard, with a mass width of 0.6–0.7, amu (low resolution), a dwell time of 439 ms, and a 100 V fragmentor potential.

The concentration range of the calibration standards of BMS-214662 (methanesulfonate) in human plasma was 1.2–60 ng/ml. Study samples were independently assayed in duplicate, on separate days, together with a series of calibration standards and a set of three quality control samples of the drug in human plasma (50, 20, and 3.6 ng/ml). Chromatograms were integrated to provide peak areas. Standard curves were constructed by plotting the drug/internal standard chromatographic peak area ratio against the known drug concentration in each calibration standard. Linear least squares regression was performed with weighting in proportion to the reciprocal of the drug concentration normalized to the number of calibration standards. Values of the slope and the y-intercept of the best-fit line were used to calculate the drug concentration in study samples. Specimens with concentrations exceeding the upper range of the standard curve were reassayed upon appropriate dilution with drug-free human plasma. The average of the two determinations of each study sample was calculated. Samples were reassayed in cases where the individual determinations differed from their average by more than 10%.

Retention times were typically 4.9 min (0.27 min peak width at half-height) for BMS-214662 and 8.9 min (0.39 min peak width at half-height) for the internal standard. Peaks that interfered with the detection of the drug or internal were not evident in any chromatogram of drug-free plasma from more than six anonymous donors, pretreatment plasma obtained from cancer patients participating in this clinical trial, and plasma samples obtained from patients during and after the infusion of BMS-214662. Accuracy of the assay for measuring 15 independently prepared sets of quality control samples with added BMS-214662 (methansulfonate) concentrations of 3.64, 20.0 and 50.1 ng/ml over a 6 month period ranged from 97.8 to 100.6% and the precision was 8.2–8.6%. Accuracy and precision for measuring BMS-214662 at the 1.2 ng/ml lower limit of quantitation were 109.8 and 4.1%, respectively.

Actual sample times were calculated from the beginning of the drug infusion to the sample collection time. FT activity was normalized to the total protein concentration in the cytosolic extract and values are reported as the percentage of activity in the pretreatment sample. Assayed values of the BMS-214662 concentration in study samples were converted to the free base equivalent concentration and are reported as such. Individual patient plasma concentration–time curves of BMS-214662 (free base) were analyzed by standard non-compartmental methods using routines provided in the WinNonlin Professional version 4.0.1 software package (Pharsight Corp., Cary, NC, USA) [7]. The area under the plasma concentration–time curve (AUC) was estimated using the logarithmic-linear trapezoidal algorithm to the last data point, with extrapolation to time infinity using the estimated value of the slope of the terminal logarithmic-linear disposition phase. Estimated values of the pharmacokinetic parameters at each dose level are reported as the geometric mean ± standard deviation (SD) of the values for the individual patients [8, 9]. SDs for the geometric mean values were estimated by the jacknife method [10]. Parametric statistical tests of pharmacokinetic variables were performed after logarithmic transformation of the data. All tests were two-sided and a value of $P < 0.05$ was the criterion for significance.

Results

Patient characteristics

Characteristics of the 19 patients enrolled into the study and treated with BMS-214662 are given in Table 1. These values are representative of the patient population enrolled into similar trials at our institutions. The duration of the study was from August 2000 until May 2002.

Determination of the MTD

Clinical observations of the patients treated at each dose level evaluated in establishing the MTD are presented in Table 2. Single patients received a dose of 56 mg/m^2 given as a 2 and 4 h i.v. infusion with no treatment-related toxicities. (The grade 3 pneumonitis experienced by patient 1 was not treatment-related.) Two patients received this same dose when infused over 8 h and also at 16 h because the initial patients were inevaluable. An apparent failure in the operation of the ambulatory infusion pump used for treating the first patient who was scheduled to receive an 8 h infusion resulted in delivering the dose in a time that was significantly less than intended. The first patient treated with a 16 h infusion of the drug developed a small bowel obstruction due to progressive colon cancer that required surgery before the end of the first cycle of therapy. Dose escalation as a weekly 24 h i.v. infusion for 3 weeks followed by 1 week of rest proceeded after the starting dose was given to one patient in this manner without any evidence of toxicity. Serum ALT and AST frequently became elevated for 48–72 h

Table 1 Patient characteristics

Characteristic	No. of patients
Age (years)	
Median	54.5
Range	42–64
Gender	
Male	11
Female	8
Performance status	
0	5
1	12
2	2
Primary tumor site	
Colorectal	8
Paraganglioma	1
Sarcoma	4
Lung, non-small cell	1
Pancreatic	2
Ovarian	1
Bladder, transitional cell	1
Small bowel, adenocarcinoma	1
Prior chemotherapy regimens	
0–2	8
≥3	11
Prior radiotherapy	5

after completing each weekly infusion, occasionally by fivefold or greater at maximum. This was similar to observations made following administration of the drug as a 1 h infusion [5]. These elevations resolved within a week, with one exception, that being a patient who subsequently acknowledged heavy intake of ethyl alcohol, in which case retreatment with BMS-214662 was delayed for 1 week together with complete abstention from alcohol.

The weekly dose was increased through the first nine cohorts through 215 mg/m^2 without any drug-related toxicity more severe than grade 1 [resolution of abnormalities in serum ALT and AST of any grade that resolved in 72 h or less were not considered as toxicities since these were expected (5)]. The toxicities observed were predominantly constitutional symptoms such as fatigue, lethargy and anorexia that were not completely distinguishable from those symptoms commonly associated with advanced cancer. The occurrence of a DLT in the first patient treated at 300 mg/m^2, grade 3 hyponatremia in the setting of carcinomatous ascites and edema, required expansion of the cohort to six patients. Among the additional patients evaluated, one experienced grade 2 neuropathy and the fifth patient developed grade 3 diarrhea, azotemia, and transient grade 3 transaminase elevation. The latter patient was a 54-year-old woman with colorectal cancer. Diarrhea began to occur within 24 h after receiving the first weekly dose of BMS-214662. She became clinically dehydrated and serum creatinine increased to 3.3 mg/dl, requiring hospitalization and treatment with i.v. fluids. The diarrhea, and hepatic, and renal abnormalities resolved within a week without additional medical intervention.

In consultation with investigators at the National Cancer Institute and Bristol-Myers Squibb (the sponsors of the trial), the protocol was amended to permit evaluating toxicity in an expanded cohort of patients treated with weekly doses of 275 mg/m^2, rather than the next

Table 2 Clinical observations

Weekly dose (mg/m^2)	Infusion duration (h)	No. of patients	Total no. of cycles	Toxicity ≥ grade 2	Response
56	2	1	1	Transaminitis, grade 3 Pneumonitis, grade 3[a]	PD
56	4	1	1	None	PD
56	8	2	< 1[b]	Neuropathy, grade 2	PD
			2	None	PD
56	16	2	< 1[c]		PD
			2	None	PD
56	24	1	2	None	PD
78	24	1	1	Hypoxia, grade 3[a]	PD
110	24	1	2	Vestibular grade 2[a]	PD
154	24	1	2	None	PD
215	24	1	2	None	PD
300	24	5	1	Hyponatremia, grade 3	PD
			2	None	PD
			2	None	PD
			4	None[d]	SD
			1	Diarrhea, grade 3; renal, grade 3 Neurologic grade 2	DLT
275	24	3	1	Diarrhea, grade 3; renal, grade 4	DLT
			1	Dyspnea, grade 3[a]	PD
			2	None	PD

PD progressive disease
[a]Not considered to be drug-related
[b]Patient was unevaluable due to failure of the infusion pump during cycle 1
[c]Treatment terminated and patient removed from study due to disease associated small bowel obstruction
[d]Patient removed from study due to problems with central catheter

lower previously administered dose (215 mg/m^2), based upon experience from a similar clinical trial being performed in Europe (J. Wright and M. Cooper, personal communication). One of the three patients who received BMS-214662 at 275 mg/m^2/week, a 64-year-old man with heavily pretreated metastatic colorectal cancer, developed severe diarrhea (grade 3), dehydration, and life-threatening renal failure (grade 4 creatinine elevation) within 48 h of the first infusion. He required hospitalization, i.v. fluids, and supportive care, but recovered fully within a week. The clinical trial was closed to accrual upon further consultation with the sponsors.

This study did not complete enrollment at the presumptive MTD of 275 mg/m^2 or the next lower dose because renal toxicity at the 275 mg/m^2 in the European trial, combined with the results of this trial, gave a total of three episodes of (fully reversible) grade 3 or 4 renal failure. The investigators in both trials and the sponsors were convinced that 275 mg/m^2 was not a tolerable phase II dose. Since Bristol-Myers Squibb had a limited supply of BMS-214662 and the 24 h infusion would not have produced any increase in dose beyond that attained by the 1 h infusion, further development of the 24 h infusion was halted and efforts were directed at combination phase I trials.

Response

Whereas evidence of clinical activity was noted even at low doses when BMS-214662 was given as a 1 h infusion once every 3 weeks [5], no evidence of activity was observed using the schedule evaluated in this study. Nine of the 19 patients received only one of two planned courses of therapy either because of toxicity (four patients) or rapid disease progression (five patients). Only one patient with a spindle cell sarcoma was stable when evaluated at the end of the second cycle. Even this patient received only two subsequent courses of treatment before being removed from study without disease progression because of a superior vena cava thrombosis secondary to the central venous access device.

Pharmacokinetics

Pharmacokinetic data were obtained from all but one of the 19 patients who participated in the clinical trial. Pharmacokinetic variables could not be estimated for one patient because the difficulties in maintaining peripheral vascular access resulted in the collection of an insufficient number of samples. The mean plasma concentration–time profile of BMS-214662 for the cohort of patients treated with the MTD of 275 mg/m^2 given as a 24 h i.v. infusion is shown in Fig. 1a. The drug concentration in plasma increased rapidly during the first several hours after starting the infusion and continued to increase, but at a much slower rate, throughout the remainder of the infusion. Upon terminating the infusion, plasma levels of the drug appeared to decay biexponentially in all patients.

Mean values of the pharmacokinetic variables determined in the cohort of patients evaluated at each dose level are listed in Table 3. It should be noted that the data are limited to that from a single patient at the four intermediate dose levels, which range from 78 to 215 mg/m^2, because an accelerated titration design was used for dose escalation [6]. The maximum concentration of drug in plasma C_{max} achieved in patients receiving BMS-214662 as a 24 h infusion and the AUC in all patients were proportionate to the administered dose, as shown in Fig. 2a, b, respectively. Correlation coefficients for the linear regression analysis of these relationships were 0.63 for (C_{max}) and 0.84 for AUC. Overall mean values ($\pm$SD) of the pharmacokinetic parameters calculated for the entire cohort of patients were as follows: total body clearance (CL), 15.4 ± 8.6 l/h/m^2; steady-state apparent volume of distribution, 60.8 ± 31.8 l/m^2; half-life of the terminal disposition phase ($t_{1/2,z}$), 5.49 ± 2.65 h; and mean residence time, 3.92 ± 1.84 h. There appeared to be a moderate correlation between CL (l/h) and the body surface area, as supported by a Spearman correlation coefficient of 0.47, although statistical significance was not achieved based upon the slope of the best-fit line of the ranked data ($P = 0.051$). Correlation and subgroup analyses failed to identify any other patient-related factors that contributed to the high interpatient variability in the CL of the drug. The mean CL for the 11 male patients (17.7 ± 7.7 l/h/m^2) was not significantly different ($P = 0.20$) from that of the seven females (12.6 ± 8.0 l/h/m^2). CL and age were not significantly correlated ($r = 0.09$), which was not unexpected under consideration of the unusually narrow age range of the cohort (Table 1) and the complete absence of elderly patients (i.e., age >65 years).

Biological activity

In Fig. 1, the plasma concentration–time profile of BMS-214662 (Fig. 1a) and the FT activity–time course (Fig. 1b) are compared for the three patients treated at the 275 mg/m^2 dose level. As the plasma concentration of BMS-214662 increased to a maximum of 1.33 ± 1.01 μg/ml near the end of the 24 h infusion, the FT activity in PBMCs declined to a minimum, and gradually recovered thereafter. Although considerable variability was noted within this small group of patients, the maximum degree of FT inhibition at the apparent MTD of the 24 h i.v. infusion schedule was $47 \pm 23\%$ of the baseline activity.

Discussion

The critical role of *ras* in the transformation to and maintenance of neoplasia [11, 12] has generated substantial enthusiasm for *ras* as a target of cancer thera-

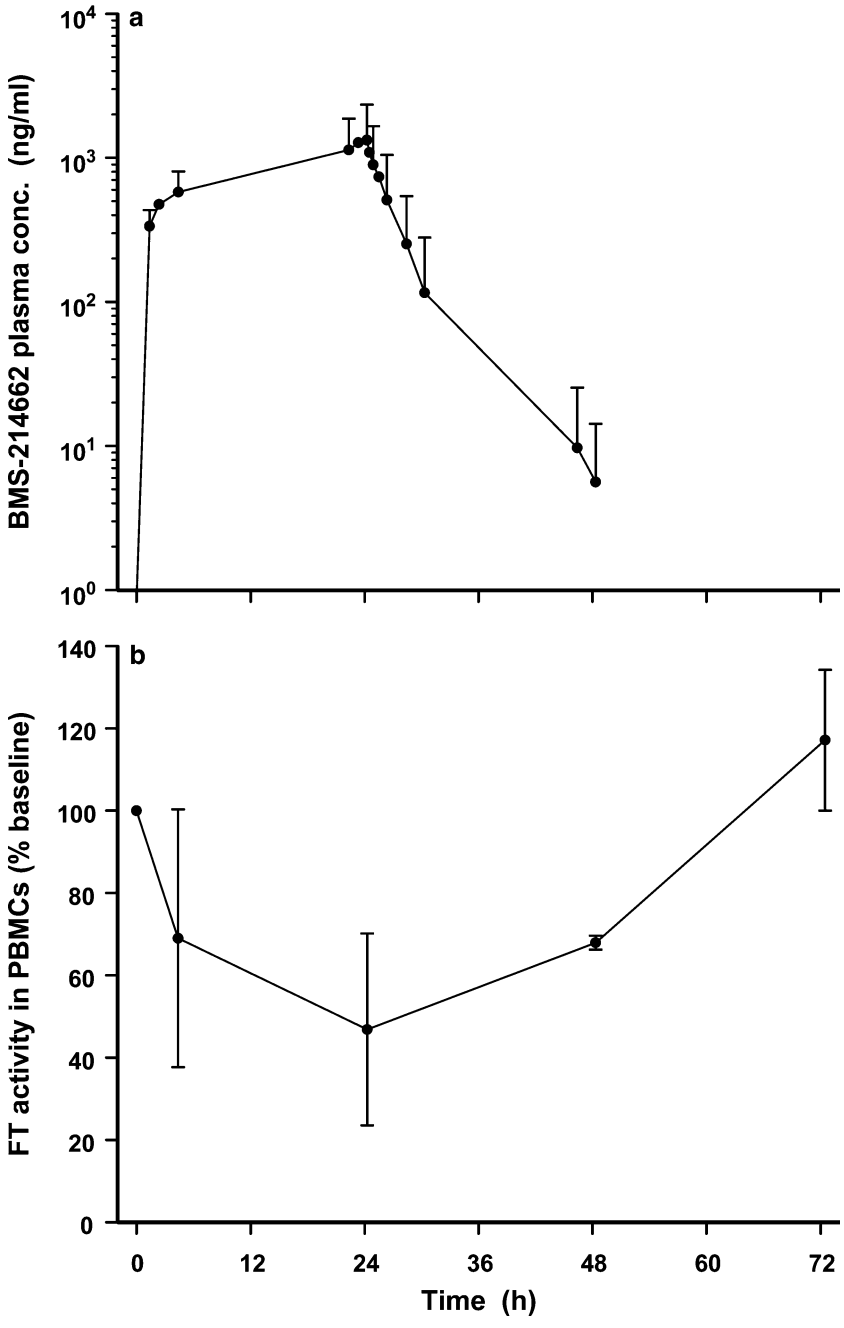

Fig. 1 **a** Mean plasma concentration–time profile of BMS-214662 for the group of patients treated with the MTD of 275 mg/m^2 given as a 24 h continuous i.v. infusion. Points (*filled circle*) are the observed plasma concentrations of the drug in each sample and the *error bars* depict the SD of the mean concentration at selected time points. **b** Time course of FT activity in PBMCs in the group of three patients treated with 275 mg/m^2 of BMS-214662. Data points represent the geometric mean value in the group of patients shown together with 1SD unit error bars

peutics. The essential requirement of farnesylation/isoprenylation to the function of the ras protein stimulated interest in inhibitors of farnesylation as potential anticancer agents. Several such compounds have entered clinical trials, including tipifarnib, lonafarnib, and BMS-214662 [13]. Each of these agents were identified during the course of screening of proprietary chemical libraries for FT inhibitory activity and bear no similarities to each other beyond being low molecular weight heterocyclic molecules that inhibit the enzyme at low nanomolar concentrations [13]. BMS-214662 is an imidazole-containing tetrahydrobenzodiazepene that is more than 1,000-fold selective for FT as compared to geranyl-geranyl transferase, inhibits both H-ras and K-ras, and produces apoptosis in cell lines with wild-type ras and in non-proliferating cells [4].

The initial phase I trial of BMS-214662, designed to evaluate its administration as a single 1 h i.v. infusion, demonstrated an acceptable safety profile and evidence of antitumor activity [5]. Results of the pharmacokinetic and pharmacodynamic studies showed that inhibition of wild-type ras farnesylation was rapidly reversible in PBMCs and that the extent of inhibition appeared to be saturated at doses below the clinical MTD or the dose recommended for phase II studies. In vitro studies performed by investigators at Bristol-Myers Squibb revealed that prolonging the duration of exposure enhanced the cytotoxic effects of BMS-214662 towards

Table 3 Mean values of BMS-214662 pharmacokinetic parameters

Dose (mg/m^2)	No. of patients	C_{max} (μg/ml)	AUC (μg h/ml)	CL (l/h/m^2)	$t_{1/2,z}$ (h)	MRT (h)	V_{ss} (l/m^2)
56	7	0.23[a]	2.67 ± 1.22[b]	21.0 ± 9.0	5.83 ± 2.11	3.09 ± 1.74	66.5 ± 47.1
78	1	0.25	4.98	15.7	9.51	4.50	70.5
110	1	0.17	3.01	36.6	4.49	3.46	126.4
154	1	1.55	26.0	5.93	7.86	6.16	36.5
215	1	0.82	16.6	13.0	1.56	2.42	31.5
275	3	1.33 ± 1.01	23.5 ± 15.1	11.7 ± 7.0	4.40 ± 1.37	5.66 ± 2.28	66.2 ± 15.1
300	4	1.29 ± 0.57	26.4 ± 10.3	11.6 ± 4.6	6.70 ± 2.75	4.53 ± 0.95	52.4 ± 15.9

C_{max} maximum plasma concentration, AUC area under the plasma concentration–time profile from time zero to infinity, CL total body clearance, $t_{1/2,z}$ half-life of the apparent terminal disposition phase, MRT mean residence time, V_{ss} apparent volume of distribution at steady-state

[a] C_{max} for the single patient receiving this dose as a 24 h i.v. infusion

[b] Geometric mean ± SD

human tumor cell lines [14]. More sustained and complete inhibition of FT may be required if inhibition is indeed essential to interrupt ras, or other farnesylated proteins, signaling through MAPK and induce apoptosis through inhibition of PI3K-AKT. These findings provided the rationale for undertaking a subsequent phase I trial to determine whether administering BMS-214662 as a 24 h continuous i.v. infusion would result in more prolonged FT inhibition and greater clinical antitumor activity than achieved with the 1 h i.v. infusion.

BMS-214662 appeared to be better tolerated in most patients when given as a 24 h continuous i.v infusion as compared to the 1 h i.v. infusion. The highest doses that did not result in severe or otherwise unacceptable toxicities were very similar for the two schedules, being 200 mg/m^2 for the 1 h infusion and 215 mg/m^2 for the 24 h infusion. However, dosing with the 24 h infusion was repeated once a week for 3 out of every 4 weeks, whereas the doses were delivered with the 1 h infusion only once every 3 weeks, resulting in a much greater weekly dose intensity for the 24 h infusion schedule. Grade 2 or 3 diarrhea, nausea and vomiting did not occur in the continuous infusion schedule within this dose range, whereas 20 such events were recorded in 22 patients receiving 200 or 225 mg/m^2 by the shorter infusion. In contrast, among the group of eight patients treated with either 275 or 300 mg/m^2 by continuous infusion, two patients developed grade 3 diarrhea within 24 h of completing the infusion that was followed by dehydration, hypotension, and non-oliguric renal failure. Tissue was not available for pathologic examination because each patient recovered fully with hydration and supportive care. There was no evidence of incremental gastrointestinal or renal problems in the other patients.

An assay based upon reversed-phase HPLC with UV detection was used to measure the drug concentration in pharmacokinetic plasma samples during our previous phase I trial of BMS-214662 given as a single i.v. infusion over 1 h [5]. Concentrations of the drug were less than 4.3 ng/ml, the lower limit of quantitation of this assay, in samples obtained 24 h after dosing from the majority (58%) of the 44 patients evaluated. The drug was consistently measurable in the preceding sample, collected 8 h after the beginning of the infusion; however, whether or not the terminal disposition phase had been achieved within this time frame was inconclusive. On the basis of this experience, it was apparent that an improvement in assay sensitivity would probably be necessary to adequately monitor the decline in plasma levels of BMS-214662 upon extending the duration of infusion from 1 to 24 h. The compound was found to be very responsive to EI-MS detection following reversed-phase HPLC. The sensitivity achieved with EI-MS detection performed in the selected-ion mode to monitor the protonated molecule allowed the lower limit of quantitation to be decreased nearly fivefold to 1.0 ng/ml (free base) using a sample volume of only 50 μl, which was ten times less sample than the previously reported assay. Using this new analytical method, the concentration of the drug in plasma samples obtained 24 h after the end of the 24 h infusion was measurable in every patient, including those treated with the starting dose in this clinical trial.

BMS-214662 exhibited apparent linear pharmacokinetics when given as a 24 h continuous i.v. infusion, as observed in the phase I trial of the 1 h infusion schedule [5]. However, there was a relatively high degree of interpatient variability in the mean pharmacokinetic parameters for the entire cohort of patients, for which the coefficients of variation ranged from 47 to 56%. Aside from the suggestion of a moderate correlation between CL and body surface area, no other patient-related factors that contributed to the high degree of variability in the CL of the drug were identified. The mean $t_{1/2,z}$ calculated from the slope of the apparent terminal log-linear phase in the plasma concentration–time profile of BMS-214662 in this study (5.49 ± 2.65 h) was notably longer than determined when the drug was given as a 1 h infusion (1.62 ± 0.40 h). This difference results from better elucidation of the terminal disposition phase through the use of a more sensitive and specific analytical method for pharmacokinetic drug level monitoring in the present study. Plasma levels of the drug decayed in a polyexponential manner, with the development of the slower, terminal disposition phase occurring 6–8 h after the end of the infusion. The effect

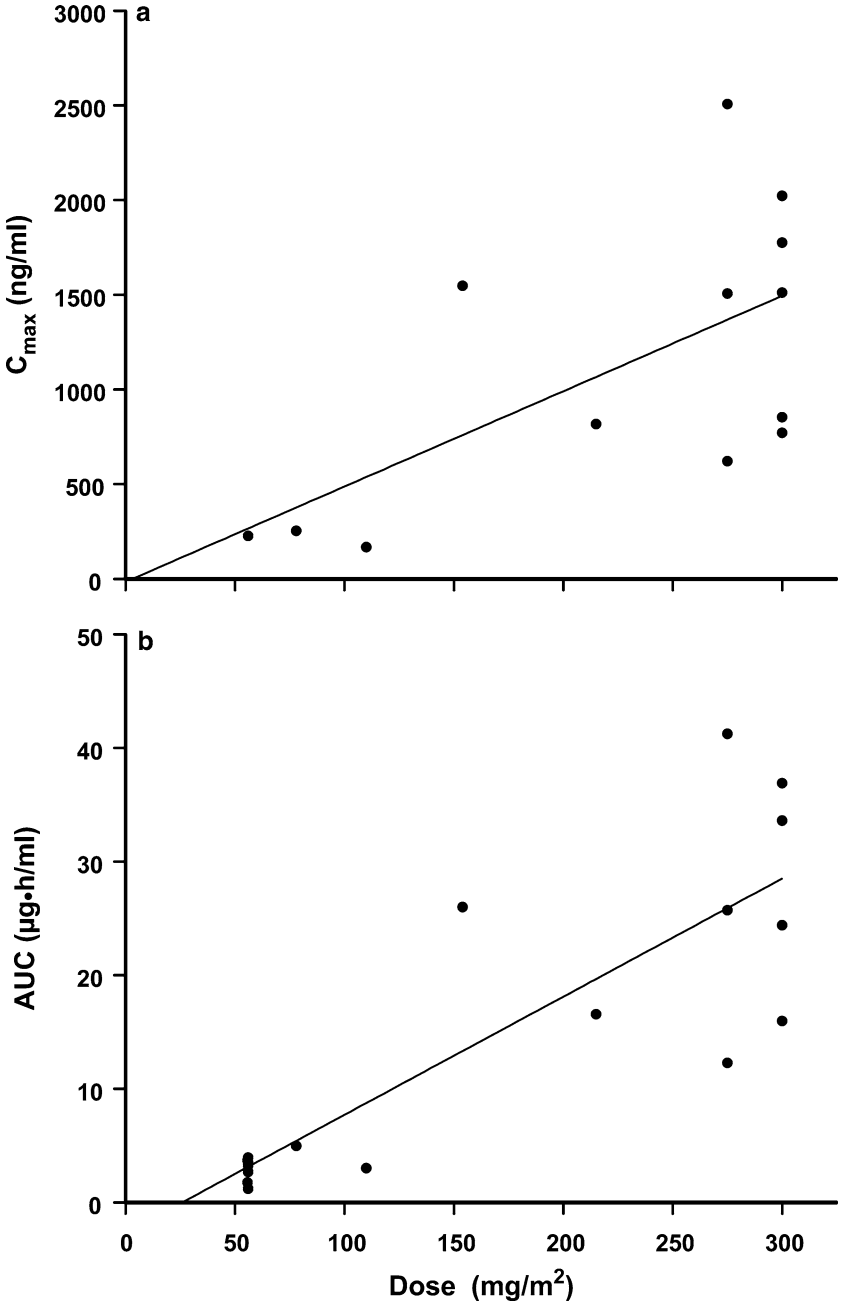

Fig. 2 Plots demonstrating the relationship between the C_{max} (**a**) and AUC (**b**) values of BMS-214662 and the dose. The plot of C_{max} as a function of dose only includes data from patients receiving the drug as a 24 h infusion. The plot depicting the relationship between AUC and dose includes data from patients treated with the starting dose given by various shorter duration infusions. The *solid lines* were generated from linear regression analysis of the observed values in individual patients (*closed circles*) at each dose

of limiting data to samples obtained through 8 h after dosing, as in our previous clinical trial of the drug, upon estimation of the $t_{1/2,z}$ and AUC was examined by analyzing the mean plasma profile shown in Fig. 1. Calculated values of the $t_{1/2,z}$ decreased from 4.3 h, when data obtained up to 24 h postinfusion were used to define the terminal phase, to only 1.8 h, which is very similar to the mean $t_{1/2,z}$ reported in the 1 h infusion study. This would result in an underestimation of the AUC, which could explain, at least in part, the comparatively greater mean CL of BMS-214662 found in the prior clinical trial (21.8 ± 10.8 l/h/m^2) than the present study (15.4 ± 8.6 l/h/m^2).

As observed when the drug was given as an i.v. infusion over 1 h, the extent of FT inhibition was greatest at the end of the infusion and was progressively reversible thereafter. In the small group of three patients treated with 275 mg/m^2 of BMS-214662, FT activity decreased to $47 \pm 23\%$ of the baseline activity in PBMCs near the end of the 24 h infusion. The enzyme remained significantly inhibited for an additional 24 h, with complete recovery of baseline activity evident in samples obtained 48 h postinfusion. In comparison, treatment with 200 mg/m^2 of the drug as a 1 h infusion decreased FT activity to a nadir $11 \pm 6\%$ of the baseline but enzyme activity was completely restored within 24 h. Thus, extending the duration of infusion from 1 to 24 h resulted in a substantial prolongation of FT inhibition in PBMCs, although the extent of maximal inhibition achieved at

doses approximating the MTDs was considerably greater for the short infusion. It is also interesting to note that the maximum degree of FT inhibition achieved at these two doses was approximately proportional to C_{max}, mean values of which were 1.3 ± 1.0 μg/ml for the 24 h i.v. infusion of 275 mg/m^2 and 6.6 ± 2.9 μg/ml for 200 mg/m^2 infused over 1 h. FT inhibition of several proteins unrelated to the ras signaling pathways occurs [15] and inhibition of one or more of these may have produced the toxicity seen in 2/8 patients treated at the highest doses in this clinical trial. The toxicity was not associated with greater than average systemic exposure to the drug in these patients.

There is no agreement on the exact mechanism by which FTinhibitors produce cytotoxicity. H-ras preferentially activates PI3K-AKT and could be cytotoxic in that genetic background but *H-ras* mutations are rare in human cancers. K-ras can become geranyl-geranylated and remain membrane bound and functional [15]. BMS-214662 is cytotoxic in the setting of a wild-type ras and it might be concluded that farnesylated proteins other than K-ras are targets of BMS-214662 and other FTIs. Wild-type ras inhibition could be important in inhibiting downstream signaling from EGFR/ERBB1 and HER2/ERBB2. The clinical trials of all the FTIs have shown little single agent activity in any solid tumor type although activity in myeloid leukemias has been observed [5, 16].

Dose intensification and prolonging the duration of exposure of the putative target to the drug are frequently employed approaches for optimizing dosing regimens of anticancer chemotherapeutic agents. Prolonging the duration of infusion from 1 to 24 h resulted in a marked increase in the dose intensity of BMS-214662 that could be delivered without a concomitant enhancement in the toxicity experienced by most patients. Unfortunately, this did not translate into an apparent improvement in the pharmacodynamic effects or therapeutic benefit of BMS-214662. Given the uncertainty of the actual therapeutic target of BMS-214662 and the multiplicity of possible collateral targets, in retrospect it is not surprising that traditional drug development strategies failed to produce the kind of results repeatedly seen with DNA-targeted cytotoxic agents. While recognizing that attempts to derive impressions of therapeutic efficacy from a phase I trial can be very misleading, the complete lack of any evidence suggestive of a potential therapeutic benefit to patients cannot be easily dismissed in view of the experience with the 1 h infusion, which suggests that the short infusion is the better schedule. One plausible interpretation is that there exists a certain threshold of FT inhibition in tumor cells necessary for growth inhibition/cytotoxicity that requires a C_{max} of the inhibitor that is greater than that which can be achieved by tolerable doses of the agent when given as a continuous infusion, although prolonged inhibition of FT at a lower level is still sufficient to produce unacceptable toxicity in normal tissues. The best approach for further clinical development of this agent appears to be selecting a different cancer type, such as myeloid disorders, or a combination with another approved or investigational chemotherapeutic agent.

Acknowledgements This study was supported in part by the grant U01-CA-62490 and the DF/HCC core grant P30-CA-0516 from the National Cancer Institute (Bethesda, MD, USA).

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