A Phase I Trial of the Novel Farnesyl Protein Transferase Inhibitor, BMS-214662, in Combination with Paclitaxel and Carboplatin in Patients with Advanced Cancer

Grace K. Dy,[1] Laura M. Bruzek,[1] Gary A. Croghan,[1] Sumithra Mandrekar,[1] Charles Erlichman,[1] Prema Peethambaram,[1] Henry C. Pitot,[1] Lorelei J. Hanson,[1] Joel M. Reid,[1] Alfred Furth,[1] Shinta Cheng,[2] Robert E. Martell,[2] Scott H. Kaufmann,[1] and Alex A. Adjei[1]

[1]Department of Oncology, Mayo Clinic and Foundation, Rochester, Minnesota and [2]Bristol Myers, Wallingford, Connecticut

ABSTRACT

Purpose: **This phase I study was conducted to determine the toxicities, pharmacokinetics, and pharmacodynamics of BMS-214662, a farnesyl transferase inhibitor, in combination with paclitaxel and carboplatin, in patients with advanced solid tumors.**

Experimental Design: **Patients with solid tumors received one of six escalating dose levels of BMS-214662 infused over 1 hour given following paclitaxel and carboplatin on the first day of a 21-day cycle. Toxicities were graded by the National Cancer Institute common toxicity criteria and recorded as maximum grade per patient for each treatment cycle. Inhibition of farnesyl transferase activity in peripheral blood mononuclear cells (PBMCs) was evaluated. Accumulation of unfarnesylated HDJ-2 in PBMCs of patients was evaluated as a marker of farnesyl transferase inhibition by BMS-214662.**

Results: **Thirty patients received 141 cycles of treatment through six dose levels. Dose-limiting toxicities were neutropenia, thrombocytopenia, nausea, and vomiting. There was no pharmacokinetic interaction between BMS-214662 and paclitaxel. The maximum tolerated dose was established as BMS-214662 (160 mg/m^2), paclitaxel (225 mg/m^2) and carboplatin (area under the curve = 6 on day 1), every 21 days. Inhibition of HDJ-2 farnesylation in PBMCs of patients was shown. One measurable partial response was observed in a patient with taxane-resistant esophageal cancer. There was partial regression of evaluable disease in two other patients (endometrial and ovarian cancer). Stable disease (> 4 cycles) occurred in eight other patients.**

Conclusions: **The combination of BMS-214662 with paclitaxel and carboplatin was well tolerated, with broad activity in solid tumors. There was no correlation between dose level and accumulation of unfarnesylated HDJ-2 in PBMCs nor tumor response.**

INTRODUCTION

Recent years have seen a flourish of mechanism-based, target-directed anticancer therapies, with multiple agents currently undergoing active clinical testing. Paclitaxel, the prototype taxane in the antimicrotubule class of cancer chemotherapeutic agents, is a cell cycle phase–specific agent that has shown impressive single-agent activity against advanced tumors of the lung, breast, esophagus, head and neck, ovary and bladder (1). This prompted its use in combination with platinum compounds such as cisplatin and carboplatin. Carboplatin is a second-generation platinum analogue that has comparable activity to, yet is less nephrotoxic and neurotoxic than cisplatin. Although it is not cell cycle–specific, cell-killing effects can be maximized if cells are in S phase upon exposure to carboplatin (2). It is currently the most commonly used platinum-containing agent in the clinical setting. The combination of paclitaxel and carboplatin has shown broad antitumor activity and is a front-line therapy in various cancers, such as non–small cell lung cancer and ovarian cancer (3).

BMS-214662 is a potent benzodiazepine-like nonthiol, nonpeptide, competitive farnesyl transferase inhibitor that has completed several phase I clinical studies (4–7). Farnesyl transferase inhibitors belong to a novel class of relatively nontoxic agents whose development was largely prompted by the pivotal role of farnesyl transferase in facilitating the membrane attachment and subsequent functioning of Ras proteins. These Ras proteins transduce upstream signals to cytoplasmic and nuclear processes, resulting in enhanced proliferation and angiogenesis as well as inhibition of apoptosis (8). However, the clinical activity of farnesyl transferase inhibitors subsequently has been shown to be independent of ras mutation status. BMS-214662 produces rapid tumor regression and cures in various human xenograft models independent of ras mutation status (9). This dose-dependent cytotoxicity is primarily attributed to induction of apoptosis even at submicromolar concentrations, with a majority of the apoptotic tumor cells arising from G_1 and S phases of the cell cycle in tumor cells with activated H-*ras* (9). Both the p.o. and i.v. forms of BMS-214662 have been evaluated in previous phase I studies, demonstrating the single-agent clinical activity of this compound. Because of considerable gastrointestinal toxicities encountered with the p.o. preparation (10), i.v. infusion has been the formulation utilized in subsequent studies.

Received 8/30/04; revised 11/23/04; accepted 12/10/04.
Grant support: Grants from the NIH (CA69912, RR00585), American Cancer Society (RSG-01-15501-CCE), and Bristol Myers Research Foundation.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Requests for reprints: Alex A. Adjei, Division of Medical Oncology, Mayo Clinic, 200 First Street, Southwest, Rochester, MN 55905. Phone: 507-538-0548; Fax: 507-284-1803; E-mail: adjei.alex@mayo.edu.

Preclinical cancer models have shown synergy between farnesyl transferase inhibitors and taxanes (11, 12). This enhanced antitumor activity is seen with both peptidomimetic and nonpeptide farnesyl transferase inhibitors, suggesting an effect of farnesyl transferase inhibitors as a class rather than related to a specific drug structure (11, 13). Moreover, it has been shown that farnesyl transferase inhibitors singly have some activity in tumors previously resistant to paclitaxel (14), and in combination with paclitaxel, can sensitize these tumors to the effect of the latter (11). The combination of BMS-214662 and paclitaxel exhibited sequence-dependent synergy in preclinical studies, when paclitaxel was given before BMS-214662 (6). Farnesyl transferase inhibitors have been reported to exhibit sequence- and cell line-dependent additive or synergistic effects when combined with platinum-based agents (15). Based on these preclinical data, we undertook a phase I trial to define the maximum tolerated dose, toxicities, pharmacokinetics, clinical activity, and farnesyl transferase inhibition after treatment combination of BMS-214662, and paclitaxel and carboplatin.

MATERIALS AND METHODS

Patient Selection. Patients with histologic or cytologic evidence of metastatic or locally advanced cancer for which no effective or proven treatment exists, or who were unresponsive to currently available therapy, and had measurable or evaluable disease, were eligible for this study. Other inclusion criteria were age ≥18 years; expected survival of at least 3 months; Eastern Cooperative Oncology Group performance status <1; adequate bone marrow (platelets $\geq 100 \times 10^9$ cells per liter, absolute neutrophil count $\geq 1.5 \times 10^9$ cells per liter; hemoglobin ≥9.0 g/dL), hepatic (total bilirubin ≤1.5 mg/dL; ALT ≤1.5 times the upper limit of reference range), and renal (stable serum creatinine ≤1.8 mg/dL) functions; no chemotherapy, radiotherapy, biologic, hormonal, or investigational drug therapy within 28 days prior to study entry; ≥6 weeks must have elapsed after nitrosourea, mitomycin C, or platinum-based chemotherapy. Patients who had significant pulmonary or cardiovascular disease; > grade 1 peripheral sensory or motor neuropathy; prior treatment with regimens requiring the use of stem cells for hematopoietic reconstitution; radiation therapy to >30% of the bone marrow; history of ≥ grade 3 hypersensitivity to paclitaxel or its vehicle; active brain metastasis; or an active infection requiring therapy were excluded from this trial. Written informed consent was obtained according to federal and institutional guidelines.

Treatment and Clinical Care of Patients. Each patient received sequential i.v. infusions of paclitaxel, carboplatin, and BMS-214662 according to the assigned dose level (Table 1). Paclitaxel was supplied in vials of either 6 mg/mL or 100 mg/17 mL formulated with mannitol and sodium acetate as a sterile lyophilized powder. This was reconstituted with normal saline to make a solution containing 10 mg/mL paclitaxel. Carboplatin was supplied as a 1 mg/mL aqueous solution in 50 and 100 mg vials for injection. The total dose was diluted in 750 mL of 5% dextrose in 1/2 normal saline containing 25 g of mannitol. BMS-214662 was supplied by Bristol-Myers in 100 mg vials containing 20 mg/mL BMS-214662 as the free base. This was diluted with 5% dextrose to a maximum concentration of 2.5 mg/mL. After appropriate premedication as prophylaxis against anaphylactoid reactions to paclitaxel or the cremophor vehicle, paclitaxel was given over a period of 3 hours. Fifteen minutes after completion of this infusion, carboplatin was given over a period of 30 minutes. The 1-hour infusion of BMS-214662 was then started 15 minutes after completing the carboplatin infusion.

Table 1 Dose escalation scheme

Dose level	*n*	BMS-214662 (mg/m^2)	Paclitaxel (mg/m^2)	Carboplatin (AUC = 6)	Cycles	Dose-limiting toxicities
1	3	80	135	6	20	—
2	6	80	175	6	30	1
3	3	120	175	6	12	—
4	5	160	175	6	31	—
5	4	160	225	6	13	—
6	9	225	175	6	35	3

BMS-214662 was given as a fixed i.v. dose weekly (days 1, 8, and 15) for the first two dose levels. Cycles were repeated every 21 days. At least three new patients were entered at each dose level in a standard "cohorts of three" phase I design (16). Dose escalation was not allowed in individual patients. However, because BMS-214662 will most likely be used in combination regimens rather than as a single agent, the study protocol was simplified by omitting the administration of BMS-214662 on days 8 and 15 from the schedule of all patients upon enrollment from the third dose level onwards. This change was likewise implemented among the few patients in the earlier dose levels who were receiving ongoing treatment at the time the protocol amendment took effect.

Complete patient histories, physical examinations, complete blood cell counts, serum electrolytes, chemistries, urinalysis, and electrocardiograms were done at baseline and prior to each course of treatment. Laboratory studies were repeated weekly while patients were on study. Ophthalmologic examination, including retinal photography, was done at baseline and prior to the third cycle of treatment. Radiologic studies (roentgenograms, computed axial tomographic scans, and magnetic resonance imaging) were done at baseline and after every two cycles of therapy to assess tumor response. A partial response required at least a 50% reduction in the sum of the products of bidimensional measurements, separated by at least 4 weeks. A complete response was defined as the disappearance of all evidence of tumor on two measurements separated by a minimum of 4 weeks. Progressive disease was the appearance of new lesion(s) or an increase in the sum of the bidimensional products of all known disease by at least 25%. Stable disease was documented when there was persistence of disease without meeting the criteria for progression, partial response, or complete response. Evaluable disease refers to lesions that could not be accurately measured in at least one dimension or whose longest diameter is <20 mm with conventional techniques of <10 mm with spiral CT scan (such as peritoneal carcinomatosis, effusions, etc.). A complete response in this situation was defined as the disappearance of all lesions and normalization of tumor marker levels. Significant regression of disease in patients with evaluable disease alone required either normalization of tumor marker level or disappearance of all visualizable lesions in the presence of tumor marker levels above the normal limit.

Dose-Limiting Toxicity. All toxicities were graded according to the National Cancer Institute common toxicity criteria (version 2.0). The maximum tolerated dose was defined as one dose level below the dose that induced dose-limiting toxicities in more than one-third of patients (at least two of a maximum of six patients). Severe or life-threatening National Cancer Institute common toxicity criteria grades 3 or 4 nonhematologic toxicity (with the exception of fatigue, myalgias/arthralgias, alopecia, nausea, vomiting, grade 3 injection site reactions, and hypersensitivity reactions) were considered dose-limiting. National Cancer Institute common toxicity criteria grade 3 or 4 nausea and vomiting in patients who had received prophylactic treatment with an optimal antiemetic regimen was considered dose-limiting. An absolute neutrophil count of $<0.5 \times 10^9$ cells per liter associated with fever or lasting for more than 5 consecutive days, a platelet count of $< 25 \times 10^9$ cells per liter of any duration or between 25 and 50×10^9 cells per liter associated with hemorrhage requiring blood transfusion, and treatment delay of more 1 week because of failure of adequate recovery from the previous cycle were also considered dose-limiting.

Blood Sampling. Plasma pharmacokinetics of BMS-214662 and paclitaxel, when given in combination with carboplatin, was evaluated during the first cycle. Fourteen blood samples per patient were collected, prior to and serially after the administration of paclitaxel within the first 24 hours. Peripheral blood mononuclear cells (PBMCs) were collected from blood samples serially in the first 24 hours in order to characterize farnesyl transferase enzyme inhibition as well as the feasibility of using unfarnesylated HDJ-2 protein as a surrogate marker of farnesyl transferase inhibition.

Pharmacokinetics. Noncompartmental pharmacokinetic analysis methods were applied to paclitaxel and BMS-214662. Area under the plasma concentration-time curve was calculated using the linear trapezoidal method. Plasma clearance was calculated by dividing dose (1,000 mg/m^2) by the area under the curve. The terminal phase rate constant (λ_z) was calculated as the negative of the slope of the log-linear terminal portion of the plasma concentration-time curve using regression. Terminal phase half-life was calculated as $0.693/\lambda_z$. Apparent steady-state volume of distribution was calculated using standard noncompartmental first-moment theory method. Data obtained for paclitaxel given alone were compared with those given in the presence of BMS-214662.

Farnesyl Transferase Enzyme Inhibition Direct Measurement. PBMCs were isolated by centrifugation in Accuspin-Histopaque tubes, depleted of RBC by distilled water lysis, pelleted, and frozen for subsequent analysis. To prepare for the simultaneous analysis of the frozen pellets, 0.2 mL buffer [20 mmol Tris-HCl (pH 7.5), plus 1 mmol DTT, and 20 μmol/L $ZnCl_2$, supplemented with 1 mmol phenylmethylsulfonyl fluoride and Calbiochem protease inhibitor cocktail; (Calbiochem, San Diego, CA)] was added. The mixture was then sonicated for 20 seconds, and centrifuged at $300,000 \times g$ for 20 minutes in a Beckman microultracentrifuge (Palo Alto, CA). Aliquots of supernatant were assayed for protein (17) and adjusted to 0.5 mg/mL (10 μg/20 μL). Farnesyl transferase activity was measured using a scintillation proximity assay kit (Amersham Pharmacia Biotechnology, Piscataway, NJ). Triplicate 20 μL samples were incubated for 60 minutes at 37°C with exogenous [^{3}H] farnesyl PPi and biotinylated H-*ras* substrate. The resulting farnesylated peptide was recovered using streptavidin-conjugated scintillant-containing beads and quantitated as described by the supplier.

Gel Electrophoresis in Peripheral Blood Mononuclear Cells. HDJ-2 is a chaperone protein that undergoes farnesylation (18), the inhibition of which is reflected in mobility shifts during electrophoresis. It has been previously shown that this protein can serve as a useful marker of farnesyl transferase enzyme inhibition in clinical samples (19). Peripheral blood, obtained serially prior to therapy, 6 and 24 hours after the infusion of BMS-214662, was cooled to 4°C. Mononuclear cells were isolated by Ficoll-Hypaque sedimentation and washed with buffer A [RPMI 1640 medium containing 10 mmol Hepes (pH 7.4), at 4°C]. Aliquots were removed for cell counts and preparation of Wright-stained cytospins for morphologic examination. Cell lysates were then prepared as previously described in detail (19). Aliquots containing 50 μg of protein (assayed by the bicinchoninic acid method; ref. 20) were subjected to electrophoresis on SDS-polyacrylamide gels containing 8% (w/v) acrylamide, transferred to nitrocellulose, and probed with monoclonal anti-HDJ-2 from Neomarkers (Fremont, CA) as described in detail (19). Antigen-antibody complexes were detected using peroxidase-coupled secondary antibodies and ECL enhanced chemiluminescence reagents. With each batch of samples, A549 lung cancer cells treated with BMS-214662 or diluent were included as positive and negative controls, respectively.

RESULTS

Patient Demographics. Thirty patients (Table 2) received a total of 141 cycles of therapy through six dose levels. Six patients received ≤2 cycles each of therapy: three experienced dose-limiting toxicities, two had progressive disease, and one died 4 days after the second cycle of unclear cause. Dose-limiting toxicities observed in dose level 6 include profound neutropenia associated with septic shock which was seen in one patient who was subsequently taken off therapy, and common toxicity criteria grade 3 nausea and vomiting. The median number of cycles given per patient was 4 (range 1-13). Eleven patients completed ≥5 cycles of therapy. The median age of study participants was 58 (range, 30-80 years). There were 17 males and 13 females enrolled. Patients had a good performance status (17 PS = 0, 13 PS = 1). Nineteen patients had received prior chemotherapy and eight had received prior radiation therapy. The most common tumor type was lung cancer, with nine patients. There were four patients with esophageal adenocarcinoma, three with breast cancer, and two patients each with mesothelioma, soft tissue sarcoma, and ovarian cancer.

Toxicities

Hematologic Toxicity. The hematologic effects of BMS-214662 in combination with paclitaxel and carboplatin and the number of patients experiencing various grades of toxicity are shown in Fig. 1*A*. Neutropenia was the most common dose-limiting toxicity. Out of 141 treatment courses, neutropenia of varying severities were seen in 77 courses, 49 (64%) of which were severe (grades 3 and 4). Twenty-five patients had some degree of neutropenia along the course of therapy. Grade 4

Table 2 Patient demographics

Patient characteristics	(n = 30)
No. of cycles	141
Median no. of cycles/patient (range)	4 (1-13)
Median age, y (range)	58 (30-80)
Gender (male/female)	17:13
Eastern Cooperative Oncology Group performance status	
0	17
1	13
2	–
Prior surgery	10
Prior chemotherapy	19
Prior radiation	8
Tumor type	
Non–small cell lung cancer	9
Esophagus	4
Mesothelium	2
Ovary	2
Soft tissue	2
Endometrium	1
Prostate	1
Breast	3
Head and neck	1
Kidneys	1
Unknown	2
Melanoma	1
Gallbladder	1

neutropenia occurred in 60%, 75%, and 78% of patients in dose levels 4, 5, and 6, respectively, compared with 0%, 50%, and 0% for dose levels 1 to 3. Thrombocytopenia, which was not as frequent, occurred in 35 treatment courses and was severe in 4 (11%). Severe anemia (1 of 29, 6%) was less common (Fig. 1*A*).

Gastrointestinal Toxicity. Diarrhea (common toxicity criteria grades 1 and 2) was common, observed in over half of all patients (Fig. 1*B*). This was largely related to BMS-214662, having been reported in prior phase I single-agent studies (4, 10). Anorexia, nausea, and vomiting, mostly common toxicity criteria grades 1 and 2, were also frequently reported symptoms (20 of 30, 24 of 30, 14 of 30 patients, respectively) and occurred at all dose levels. The dose of carboplatin was fixed throughout the six dose levels (see Table 1). These findings therefore suggest that BMS-214662 did not contribute substantially to the known emetogenic effects of carboplatin. In most instances, nausea and vomiting were controlled with aggressive prophylaxis with granisetron and dexamethasone. Whereas the severity and frequency of nausea and vomiting were not clearly related to the dose level, one patient at dose level 6 had severe grade 3 symptoms in spite of optimal antiemetic premedication. Dose-related BMS-214662-induced elevation in liver enzymes has been variably reported in earlier studies (4, 5), but was not seen in this cohort of patients.

Constitutional Symptoms. Fatigue, arthralgias, and myalgias were also common symptoms that were typically mild to moderate in severity. These were attributable to paclitaxel and carboplatin. One patient (dose level 1) had grade 3 fatigue that required withdrawal from the study.

Other Toxicities. Alopecia was universal. Mild to moderate peripheral sensory neuropathy with a glove-and-stocking distribution was reported by approximately two thirds of the patients. These were expected with respect to the standard agents used in this regimen. Mild rash was seen in a few patients (5 of 30). Based on the toxicities that precluded further dose escalation, the maximum tolerated dose was established as BMS-214662 (160 mg/m^2), paclitaxel (225 mg/m^2) and carboplatin (area under the curve = 6 on day 1), every 21 days.

Antitumor Activity. Four out of 30 patients were withdrawn from therapy after ≤ 2cycles as previously mentioned, prior to initial assessment for antitumor activity (Table 3). One partial response was observed after the fourth cycle of treatment in a patient with metastatic esophageal adenocarcinoma previously treated with two cycles of CPT-11 and docetaxel. He was enrolled at dose level 1 of this combination and received a total of 13 cycles. His intra-abdominal disease showed continued disease response immediately prior to the 13th cycle, although the presence of brain metastases was documented shortly thereafter. Significant regression of evaluable disease occurred in two patients. Both were enrolled at dose level 2. The first one was seen in a patient with endometrial cancer whose disease progressed earlier through a cisplatin- and paclitaxel-containing regimen. This response was maintained for five additional cycles after the demonstrable response was first documented in the second cycle. The second was observed in a patient with ovarian cancer who had a relapse a year after completing six cycles of adjuvant chemotherapy with carboplatin and paclitaxel. After one cycle of chemotherapy at dose level 2, her CA 125 levels normalized. There was demonstrable regression of the peritoneal implants radiographically as well. She completed eight cycles of treatment. Her response was sustained for 11 months after elective cessation of therapy. Eight other patients had stable disease for > 4 cycles of treatment.

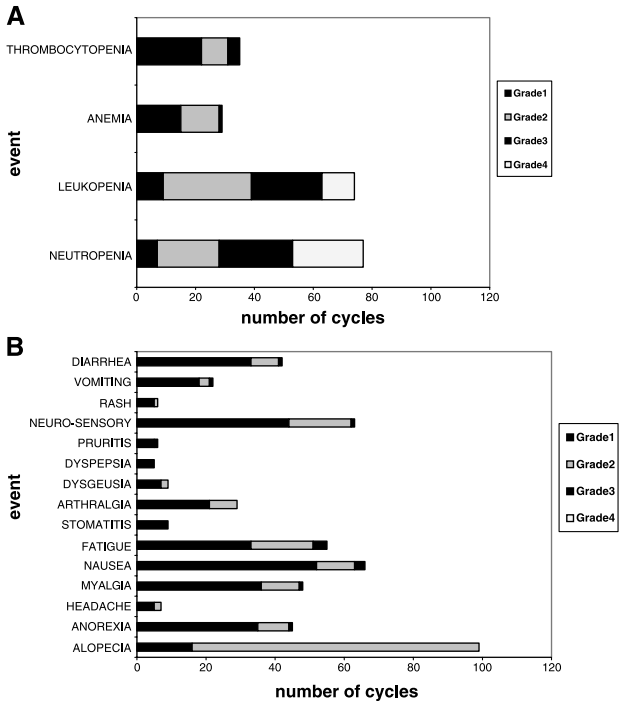

Fig. 1 Cumulative frequency of toxicities in all cycles. *A,* hematologic toxicities; *B,* nonhematologic toxicities. The number of cycles indicated represents the cumulative number of treatment cycles for all dose levels.

Table 3 Clinical activity

Best response	Primary tumor site (*n*)	Previous exposure*		Cycles given
		Cisplatin	Taxane	
Partial	Esophagus (1)	N	Y	13
Regression of evaluable disease	Ovary (1)	Y	Y	8†
	Endometrial (1)	Y	Y	7
Disease stabilization	Leiomyosarcoma (1)	N	N	5
	Esophagus (2)	N, N	N, Y	5, 5
	Lung (3)	N, N, N	N, N, N	5, 11, 8
	Melanoma (1)	N	N	9
	Breast (1)	N	Y	5

*Y = previous exposure; N = no previous exposure.
†Response sustained over 11 months after last cycle of therapy.

Inhibition of Farnesyl Transferase in Peripheral Blood Mononuclear Cells. The effect of BMS-214662 on farnesyl transferase activity was assessed by measuring activity in extracts from isolated PBMCs. Farnesyl transferase enzyme activity reached a nadir immediately at the end of the BMS-214662 infusion. The degree of farnesyl transferase enzyme inhibition was dose-dependent (Spearman correlation of dose and farnesyl transferase enzyme activity was -0.81; $P = 0.0001$) and reversible, with full recovery of enzyme activity by the 20th hour relative to the start of BMS-214662 administration (Fig. 2*A*). Immunoblot assays (Fig. 2*B*) revealed a general trend towards accumulation over time of unfarnesylated HDJ-2 protein which seemed to persist longer in patients receiving a higher dose of BMS-214662. However, unfarnesylated HDJ-2 was present in certain samples even prior to the administration of the farnesyl transferase inhibitor (Fig. 2*B*).

Pharmacokinetics

The potential for a pharmacokinetic interaction between paclitaxel and BMS-214662 was determined. Regardless of the dose of BMS-214662, plasma concentrations of paclitaxel achieved during infusion were not altered by administration of this farnesyl transferase inhibitor (Fig. 3*A*).

Steady-state plasma concentrations of BMS-214662 increased as dose increased from 80 to 225 mg/kg (Fig. 3*B*). Plasma clearance and $t_{1/2}$ were not significantly changed at the various dose levels, confirming linearity of BMS-214662 pharmacokinetics over this dose range as well as the apparent absence of interaction with paclitaxel.

For paclitaxel, no significant difference between any pharmacokinetic estimate was seen when comparing with and without BMS-214662 administration.

DISCUSSION

The evaluation and clinical use of farnesyl transferase inhibitors as a class continues to evolve as a promising small molecule inhibitor of dysregulated cell signaling in malignancies. The pharmacology and clinical activity of the farnesyl transferase inhibitors have been comprehensively reviewed recently (21–25). Like other specific target–directed agents, farnesyl transferase inhibitors may be more effective in combination with cytotoxic chemotherapy because of the heterogenous and multistep nature of carcinogenesis. Preclinical studies have shown sequence-dependent cytotoxic synergy when cultured human cancer cell lines were exposed to paclitaxel and farnesyl transferase inhibitors *in vitro* (6). In addition, synergy between farnesyl transferase inhibitors and cisplatin has been observed *in vitro* (15). Multiple regimens combining various farnesyl transferase inhibitors and platinum or taxane analogues have been tested (7, 26–28). In this phase I trial, we studied the safety and efficacy of BMS-214662 in combination with one of the most effective combination regimens in wide clinical use. The maximum tolerated dose was determined to be BMS-214662 (160 mg/m^2) infused over 1 hour, paclitaxel (225 mg/m^2) and carboplatin (area under the curve = 6 on day 1), every 21 days.

Overall, the toxicities observed were expected from the known effects of the individual agents in this combination. Neutropenia was the main dose-limiting adverse effect. The severity of neutropenia seemed to be dependent on the dose level of BMS-214662 in addition to that contributed by the combination of paclitaxel and carboplatin in general.

It has been shown that the processing of certain proteins, such as prelamin A or HDJ-2, in easily obtainable tissue specimens from patients could be utilized as a surrogate marker of farnesyl transferase activity *in vivo* (18). The degree of expression of unfarnesylated HDJ-2 protein in PBMCs is

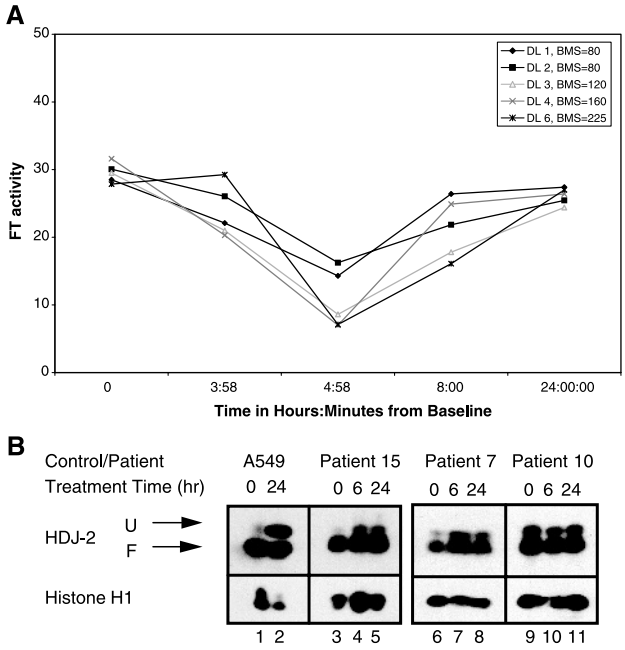

Fig. 2 Pharmacodynamic markers of BMS-214662 activity in PBMCs. *A,* median farnesyl transferase activity in PBMCs over time per dose level showing dose-dependent reversible farnesyl transferase enzyme inhibition. Time in hours/minutes relative to the onset of paclitaxel infusion. 3:58 is the time point at the end of the carboplatin infusion, before the start of the BMS-214662 infusion. 4:58 is the time point at the end of the BMS-214662 infusion. *B,* inhibition of HDJ-2 farnesylation in peripheral blood mononuclear cells. Peripheral blood mononuclear cells were prepared and processed for SDS-PAGE immunoblotting with anti-HDJ-2 antibody are described in Materials and Methods. *F,* farnesylated HDJ-2; *U,* unfarnesylated HDJ-2. Anti Histone H1 antibody was used as a loading control. A549 cells treated with 1 μmol/L BMS-214662 or vehicle for 24 hours serve as positive and negative controls.

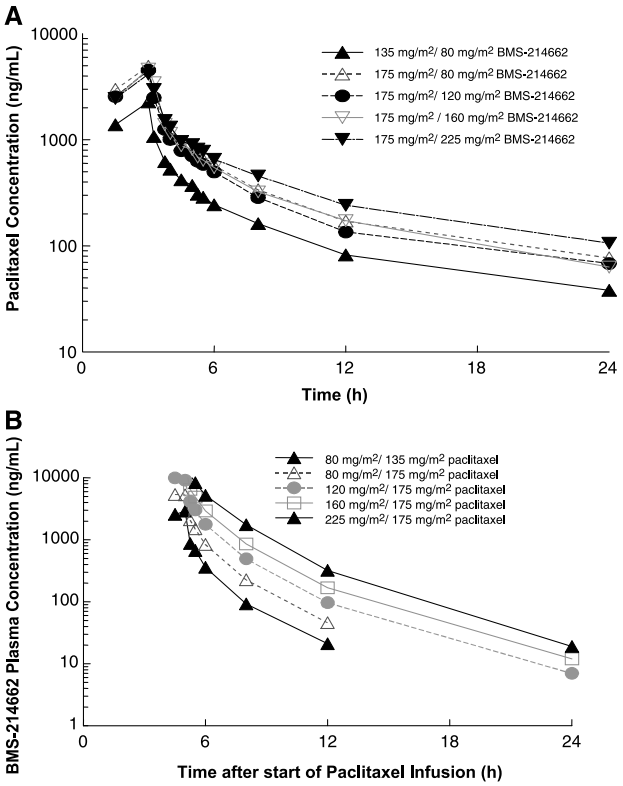

Fig. 3 Mean plasma concentration-time profile (*A*) of paclitaxel in combination with carboplatin and escalating doses of BMS-214662 given as a 1-hour i.v. infusion (*B*) of escalating doses of BMS-214662 given as a 1-hour i.v. infusion after paclitaxel and carboplatin.

proportional to the dose level of BMS-214662 given in most cases, however, this is not statistically significant. On the other hand, the unexpected presence of unfarnesylated HDJ-2 prior to infusion of BMS-214662 was observed in samples from some patients, regardless of dose level and degree of farnesyl transferase enzyme activity at baseline. This observation might reflect the heterogeneity of farnesyl transferase enzyme affinity for the protein substrate, in this case, HDJ-2. Further study is required to determine whether accumulation of surrogate markers correlates with antitumor activity.

In contrast with the results from an earlier published phase I trial of BMS-214662 in combination with cisplatin where no objective responses were seen (7), the current regimen was found to be potentially active in several tumors, including those progressing on prior platinum- and taxane-based regimens. There was one confirmed objective response documented after the fourth cycle in a patient who had a taxane-refractory esophageal adenocarcinoma. He was enrolled in dose level 1 and thus received weekly BMS-214662 initially (for five cycles, prior to protocol modification), maintaining this response for nine cycles. Significant regression of evaluable disease occurred in two patients, one of whom had progression of endometrial cancer that had previously progressed through a cisplatin- and paclitaxel-containing regimen. This ability to sensitize tumors to taxane effects and reverse potential resistance (11, 12) may be attributed to the effects of farnesyl transferase inhibitor on cell cycle arrest (29) and inhibition of P-glycoprotein (30).

In this study, we confirmed that BMS-214662 inhibited farnesyl transferase enzyme in a dose-dependent fashion in clinical samples. This inhibition was reversible within 24 hours, and confirms the earlier results of Mackay et al. (7). Nevertheless, it is important to note that a biological threshold of farnesyl transferase inhibition that can be utilized as a surrogate marker to correlate with clinical outcome has not been established. In contrast to other farnesyl transferase inhibitors that are given p.o. daily, differences in pharmacodynamic effects may in part be schedule-dependent as BMS-214662 was delivered intermittently at longer intervals. Although the optimal timing of farnesyl transferase inhibition within tumors for antitumor effect and synergy with taxanes and/or platinum agents is not well elucidated, and the clinical relevance of prolonged farnesyl transferase inhibition in normal tissues is unclear, weekly schedules of administration may provide a more consistent plasma exposure as well as a more sustained pharmacodynamic effect that may be required for optimal activity. It is interesting to note that the two patients in our study who had tumor responses were among the five patients who received weekly BMS-214662 prior to protocol modification.

Many of the cases of disease stabilization that occurred in non–small cell lung cancer were in either taxane- or platinum-naïve patients. Moreover, the patient with ovarian cancer who achieved both radiographic regression of peritoneal disease and tumor marker (CA-125) normalization had platinum-sensitive disease. The antitumor activity reported in this study thus has to be interpreted with caution for two reasons. First, this study represents a combination of a farnesyl transferase inhibitor with a regimen that by itself is clinically effective. Second, the trial was not designed to evaluate the efficacy of antitumor activity of this combination.

In conclusion, this study has shown that clinically relevant doses of all three agents can be safely combined to treat patients. Not only is the BMS-214662 dose effective in inhibiting farnesyl transferase, but the paclitaxel and carboplatin doses recommended for future studies are also within the therapeutic range. Because the antitumor activity of BMS-214662 is dependent on the cumulative dose given other than the schedule used (9), re-examining the feasibility of its weekly administration might be considered.

ACKNOWLEDGMENTS

We thank Sue Steinmetz and Melanie Kassner for excellent data management, Sara Felten for statistical programming support, Sacha Nelson for protocol management, and Raquel Ostby for secretarial assistance.

REFERENCES

1. Rowinsky EK. The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. Annu Rev Med 1997;48:353–74.

2. Nguyen HN, Sevin BU, Averette HE, et al. Cell cycle perturbations of platinum derivatives on two ovarian cancer cell lines. Cancer Invest 1993;11:264–75.

3. Alberts DS, Dorr RT. New perspectives on an old friend: optimizing carboplatin for the treatment of solid tumors. Oncologist 1998; 3:15–34.

4. Ryan DP, Eder JP, Puchlaski T, et al. Phase I clinical trial of the farnesyltransferase inhibitor BMS-214662 given as a 1-hour intravenous

infusion in patients with advanced solid tumors. Clin Cancer Res 2004;10:2222–30.

5. Voi M, Tabernero J, Cooper MR, et al. A phase I study of the farnesyltransferase (FT) inhibitor BMS-214662 administered as a weekly 1-hour infusion in patients (pts) with advanced solid tumors: clinical findings [abstract 312]. Proc Am Soc Clin Oncol 2001;20.

6. Bailey HH, Marnocha R, Arzoomanian R, et al. Phase I trial of weekly paclitaxel and BMS214662 in patients with advanced solid tumors [abstract 314]. Proc Am Soc Clin Oncol 2001;20.

7. Mackay HJ, Hoekstra R, Eskens FA, et al. A phase I pharmacokinetic and pharmacodynamic study of the farnesyl transferase inhibitor BMS-214662 in combination with cisplatin in patients with advanced solid tumors. Clin Cancer Res 2004;10:2636–44.

8. Adjei AA. Blocking oncogenic Ras signaling for cancer therapy. J Natl Cancer Inst 2001;93:1062–74.

9. Rose WC, Lee FY, Fairchild CR, et al. Preclinical antitumor activity of BMS 214662, a highly apoptotic and novel farnesyltransferase inhibitor. Cancer Res 2001;61:7507–17.

10. Camacho LH, Soignet SL, Pezzulli S, et al. Dose escalation study of oral farnesyl transferase inhibitor (FTI) BMS-214662 in patients with solid tumors [abstract 311]. Proc Am Soc Clin Oncol 2001;20.

11. Shi B, Yaremko B, Hajian G, et al. The farnesyl protein transferase inhibitor SCH66336 synergizes with taxanes *in vitro* and enhances their antitumor activity *in vivo*. Cancer Chemother Pharmacol 2000;46: 387–93.

12. Moasser MM, Sepp-Lorenzino L, Kohl NE, et al. Farnesyl transferase inhibitors cause enhanced mitotic sensitivity to taxol and epothilones. Proc Natl Acad Sci U S A 1998;95:1369–74.

13. Sun J, Blaskovich MA, Knowles D, et al. Antitumor efficacy of a novel class of non-thiol-containing peptidomimetic inhibitors of farnesyltransferase and geranylgeranyltransferase I: combination therapy with the cytotoxic agents cisplatin, taxol, and gemcitabine. Cancer Res 1999;59:4919–26.

14. Petit T, Izbicka E, Lawrence RA, Bishop WR, Weitman S, Von Hoff DD. Activity of SCH 66336, a tricyclic farnesyltransferase inhibitor, against human tumor colony-forming units. Ann Oncol 1999;10:449–53.

15. Adjei AA, David JN, Bruzek LM, Erlichman C, Kaufmann SH. Synergy of the protein farnesyltransferase inhibitor SCH66336 and cisplatin in human cancer cell lines. Clin Cancer Res 2001;7:1438–45.

16. Ratain MJ, Mick R, Schilsky RL, Siegler M. Statistical and ethical issues in the design and conduct of phase I and II clinical trials of new anticancer agents. J Natl Cancer Inst 1993;85:1637–43.

17. Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

18. Neckers L, Mimnaugh E, Schulte TW. The Hsp90 chaperone family. In: Latchman DS, editor. Stress Proteins. New York: Springer-Verlag; 1999. p. 9–42.

19. Adjei AA, Davis JN, Erlichman C, Svingen PA, Kaufmann SH. Comparison of potential markers of farnesyltransferase inhibition. Clin Cancer Res 2000;6:2318–25.

20. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76–85.

21. Mazieres J, Pradines A, Favre G. Perspectives on farnesyl transferase inhibitors in cancer therapy. Cancer Lett 2004;206:159–67.

22. Sebti SM, Der CJ. Searching for the elusive targets of farnesyltransferase inhibitors. Nature Rev Cancer 2003;3:945–51.

23. Haluska P, Dy GK, Adjei AA. Farnesyl transferase inhibitors as anticancer agents. Eur J Cancer 2002;38:1685–1700.

24. Sebti SM, Adjei AA. Farnesyltransferase inhibitors. Sem Oncol 2004;31:28–39.

25. Adjei AA. Farnesyltransferase inhibitors. In: Giaccone G, Schilsky R, Sondel P, editors. Cancer chemotherapy and biologic response modifiers. Annual 21. Amsterdam: Elsevier Science BV; 2003. p. 127–44.

26. Adjei AA, Croghan GA, Erlichman C, et al. A phase I trial of the farnesyl protein transferase inhibitor R115777 in combination with gemcitabine and cisplatin in patients with advanced cancer. Clin Cancer Res 2003;9:2520–6.

27. Khuri FR, Glisson BS, Kim ES, et al. Phase I study of the farnesyltransferase inhibitor lonafarnib with paclitaxel in solid tumors. Clin Cancer Res 2004;10:2968–76.

28. Piccart-Gebhart MJ, Branle F, de Valeriola D, et al. A phase I, clinical and pharmacokinetic (PK) trial of the farnesyl transferase inhibitor (FTI) R115777 + docetaxel: a promising combination in patients (pts) with solid tumors [abstract 318]. Proc Am Soc Clin Oncol 2001;20.

29. Ashar HR, James L, Gray K, et al. The farnesyl transferase inhibitor SCH 66336 induces a G(2) → M or G(1) pause in sensitive human tumor cell lines. Exp Cell Res 2001;262:17–27.

30. Wang EJ, Johnson WW. The farnesyl protein transferase inhibitor lonafarnib (SCH66336) is an inhibitor of multidrug resistance proteins 1 and 2. Chemotherapy 2003;49:303–8.