A Phase I, Pharmacokinetic, and Biological Study of the Farnesyltransferase Inhibitor Tipifarnib in Combination with Gemcitabine in Patients with Advanced Malignancies


ABSTRACT

Purpose: To assess the feasibility of administering tipifarnib, an oral nonpeptidomimetic competitive inhibitor of farnesyltransferase, in combination with gemcitabine and recommend doses for disease-directed clinical trials. The study also sought to identify drug-drug pharmacokinetic interactions, evaluate effects on protein farnesylation, and seek preliminary evidence for clinical activity.

Experimental Design: Patients with advanced solid malignancies were treated with tipifarnib at doses of 100, 200, and 300 mg twice daily continuously and 1000 mg/m² gemcitabine i.v. on days 1, 8, and 15 every 4 weeks. To identify pharmacokinetic interactions, the treatment and plasma sampling schemes were designed to permit comparisons of the pharmacokinetic behavior of each agent administered alone and together. The proportions of unfarnesylated and farnesylated HDJ2, a chaperone protein that undergoes farnesylation, were measured in peripheral blood mononuclear cells.

Results: Nineteen evaluable patients were treated with 74 courses of tipifarnib/gemcitabine (mg/m²/m²). Myelosuppression was the principal toxicity. Dose-limiting myelosuppression occurred in 2 of 5 patients at the 300/1000 dose level, whereas 2 of 11 evaluable patients at the 200/1000 dose level experienced dose-limiting toxicity. There was no evidence of clinically relevant pharmacokinetic interactions between tipifarnib and gemcitabine. Inhibition of farnesylation of HDJ2, a potential surrogate for Ras and/or other potentially relevant farnesylated proteins, was demonstrated in peripheral blood mononuclear cells at all dose levels. Partial responses were noted in patients with advanced pancreatic and nasopharyngeal carcinomas.

Conclusions: On the basis of the results of this study, the tipifarnib/gemcitabine dose level of 200/1000 is recommended for disease-directed studies. At this dose level, biologically relevant plasma concentrations of tipifarnib that consistently inhibit protein farnesylation in vitro are achieved and drug-induced inhibition of protein farnesylation is measured in most patients.

INTRODUCTION

Ras proteins are activated downstream of receptor tyrosine kinases and trigger a cascade of phosphorylation events through sequential activation of multiple effector pathways, including the mitogen-activated protein kinase, and phosphoinositide-3-OH kinase pathways, which are critical for cell proliferation, differentiation, and survival (1–5). After synthesis as cytosolic precursors, Ras proteins undergo a series of posttranslational modifications, the first and most important being the addition of a 15-carbon farnesyl isoprenoid group catalyzed by FTase, enabling association with the cell membrane (1, 5, 6). When Ras is stimulated by receptor activation to bind GTP, it activates several downstream effectors, thereby promoting cell proliferation. The intrinsic GTPase activity of Ras then serves to return the molecule back to its inactive state. Mutations in the ras genes that are most commonly found in human malignancies

2 The abbreviations used are: FTase, farnesyltransferase; FTI, FTase inhibitor; GGTase I, geranylgeranyltransferase type I; MTD, maximum tolerated dose; BID, twice daily; ANC, absolute neutrophil count; DLT, dose-limiting toxicity; PBMC, peripheral blood mononuclear cell; HPLC, high-performance liquid chromatography.
favor Ras in its GTP-bound active state, in which it constitutively enables transmission of proliferative and survival signals (1, 2, 4, 7). Abnormal expression and conformation changes in Ras, conferred by ras gene mutations, have been demonstrated in ~30% of all human malignancies including ~50% of colorectal, 70–90% of pancreatic, and 30% of non-small cell lung cancers (7).

FTIs were developed on the premise that FTase inhibition would prevent Ras processing and, hence, transduction of critical proliferative and survival signals (8). The coupling of Ras to protein tyrosine kinases also raises the possibility that malignancies driven by overactivity upstream of Ras might be inhibited by therapeutics against this target (1, 9). Additionally, a large number of multifunctional proteins, as well as Ras, undergo farnesylation, and several of these proteins, including RhoB, Akt2, and the centromere-associated protein-E and centromere-associated protein-F centromeric proteins, seem to be affected critically by FTIs (10–13).

Tipifarnib (R115777, Zarnestra; Johnson & Johnson Pharmaceutical Research and Development, Titusville, NJ; Fig. 1), an oral quinolone analogue of imidazole-containing heterocyclic compounds, is a nonpeptidomimetic competitive inhibitor of FTase (14, 15). Tipifarnib is highly specific for FTase and does not inhibit GGTTase I, which can alternatively prenylate both K-Ras and RhoB (6, 14). Tipifarnib inhibits the growth of a broad spectrum of human malignancies in vitro and in human tumor xenografts that possess either wild-type or various types of ras mutants (6, 14–16). The feasibility of administering tipifarnib has also been evaluated in patients with advanced solid malignancies and leukemia, resulting in the characterization of the toxicity profile and pharmacokinetic behavior of the agent (17–21). Myelosuppression has been the principal DLT. The MTD of tipifarnib, when administered as a single agent on a continuous schedule, was found to be 300 mg BID (19).

The rationale for evaluating the administration of tipifarnib in combination with gemcitabine (tipifarnib/gemcitabine) includes the established role of gemcitabine in malignancies associated with high incidences of ras mutations (e.g., carcinomas of the pancreas, lung, and urothelium), the distinct spectrum of anticancer activity demonstrated with tipifarnib in preclinical studies, and the widely disparate mechanisms of action of these agents. This study was also designed to provide the toxicological and pharmacological foundation for disease-directed trials, including a Phase III study in advanced pancreas cancer (22). The objectives of this study were to: (a) characterize the principal toxicities of tipifarnib administered BID in combination with gemcitabine on a conventional dose schedule (1000 mg/m² i.v. on days 1, 8, and 15 every 4 weeks) in patients with advanced solid malignancies; (b) determine the MTD of tipifarnib in combination with gemcitabine on this dose schedule and recommend doses for subsequent disease-directed trials; (c) describe the pharmacokinetics and pharmacodynamics of tipifarnib and gemcitabine in combination and determine whether there are major effects of each agent on the clearance of the other; (d) seek preliminary evidence of antitumor activity in patients with advanced malignancies; and (e) determine whether farnesylation of HDJ2, a chaperone protein and potential surrogate for Ras and/or other critical farnesylated proteins, is inhibited at clinically relevant doses of tipifarnib and gemcitabine.

**MATERIALS AND METHODS**

Patients with histologically confirmed advanced solid malignancies refractory to standard therapy or for whom no effective therapy existed were candidates for this study. Eligibility criteria included: (a) age of ≥18 years; (b) Eastern Cooperative Oncology Group performance status ≤2 (ambulatory and capable of self-care); (c) no chemotherapy, radiotherapy, or investigational therapy within the previous 4 weeks (6 weeks for nitrosoureas or mitomycin C); (d) adequate hematopoietic (ANC ≥1,500/μl; platelets ≥100,000/μl; hemoglobin ≥9 g/dl), hepatic (total bilirubin within the institutional normal limits; aspartate aminotransferase and alanine aminotransferase ≤2.0 times the institutional upper normal limits, unless caused by hepatic metastases, in which case elevations of ≤5.0 times the upper normal limits were permitted), and renal (serum creatinine within institutional normal limits) functions; (e) no prior extensive myelotoxic therapy [defined as more than six courses of alkylating agent-containing chemotherapy (except low-dose cisplatin); more than four courses of carboplatin; two or more courses of mitomycin C or a nitrosourea; irradiation to ≥25% of hematopoietic reserves; high-dose chemotherapy, followed by hematopoietic stem cell rescue]; (f) no concurrent radiation therapy (except palliative radiation therapy), chemotherapy, hormonal therapy, or immunotherapy; and (g) no coexisting medical conditions likely to interfere with study procedures. Written informed consent was obtained according to federal and institutional guidelines.

**Dosage and Dose Escalation.** The starting dose of tipifarnib was 100 mg BID p.o. from day 2 of the first course without interruption. The dose of tipifarnib was escalated in 100-mg BID increments in successive cohorts of new patients, whereas the dose of gemcitabine was fixed at 1000 mg/m² i.v. on days 1, 8, and 15 every 28 days. A course was defined as 4 weeks in length. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0). A minimum of three new patients was treated at each dose level. If DLT was not observed in the first three patients, then the dose of tipifarnib was increased to the next level. If DLT occurred in any of the first three new patients in the first course, at least three additional new patients were treated. If no further DLT
was encountered, dose escalation proceeded. Alternately, if DLT was noted in one or more of three additional subjects, dose escalation was to be terminated, and the MTD was defined as the highest dose level at which less than one-third of at least six new patients experienced DLT in course 1. Intrapatient dose escalation was not permitted. The following events were considered dose limiting: (a) ANC < 500/μl for longer than 5 days or associated with fever (temperature, ≥38°C) or infection requiring parenteral antibiotics; (b) platelets < 50,000/μl for longer than 5 days or any platelet count < 25,000/μl; (c) any drug-related grade 3 or grade 4 nonhematological toxicity, except alopecia, nausea, and vomiting in the absence of optimal antiemetic medication, or asymptomatic elevations of hepatic transaminases thought to be secondary to gemcitabine; and (d) missing two consecutive weekly doses of gemcitabine because of unresolved toxicity.

**Drug Administration.** Tipifarnib, supplied by Johnson & Johnson Pharmaceutical Research and Development as 100-mg capsules, was administered after meals every 12 h. Gemcitabine (Gemzar; Eli Lilly and Company, Indianapolis, IN) was obtained commercially as a lyophilized powder in sterile vials containing either 200 mg or 1 g of gemcitabine as the hydrochloride salt. Gemcitabine was stored at room temperature and reconstituted with normal saline to a concentration of 40 mg/ml, which was further diluted to a final volume of 250 ml before administration over 30 min.

**Pretreatment Assessment and Follow-Up Studies.** A history, physical examination, and routine laboratory studies were performed before treatment and at least weekly during treatment. Routine laboratory studies included serum electrolytes, chemistries, renal and liver function tests, complete blood cell and differential WBC counts, coagulation studies, and urinalysis. Ophthalmological evaluations, which included an ophthalmic history and slit lamp biomicroscopy, were performed before treatment and then before each subsequent course. PBMCs were collected before treatment and weekly during the first 8 weeks of treatment. Relevant radiological studies to assess measurable and evaluable disease were repeated after every other course. Patients were able to continue treatment if they did not develop progressive disease, which was defined as an increase in the sum of the bidimensional product of measurable disease by at least 25% or the appearance of any new lesion. A complete response was scored if there was disappearance of all active disease on two measurements separated by a minimum period of 4 weeks, whereas a partial response required at least a 50% reduction in the sum of the product of the bidimensional measurements of all index lesions, separated by at least four weeks.

**Pharmacokinetic Sampling and Analytical Methodology.** To study relevant pharmacokinetic parameters reflecting both gemcitabine and tipifarnib exposure, blood was sampled during the first course of treatment. The purpose of the sampling schedule was to ascertain the pharmacokinetic information of each drug alone and in combination and specifically to determine whether there were any major effects of each drug on the clearance of the other. Whole blood samples were collected for analysis of gemcitabine plasma concentrations on day 1 at the following times: before treatment, 15 min, and at 30 min (immediately before the end of the infusion), and at 5, 15, 30, 60, and 90 min and 2, 2.5, 4.5, and 7.5 h after the end of the infusion. Daily administration of tipifarnib commenced on day 2, and on day 15, pharmacokinetic sampling of both drugs was initiated. On day 15, tipifarnib was administered at time zero and 30 min before the start of the gemcitabine infusion, and blood sampling for tipifarnib plasma concentrations was obtained before treatment and at 1, 2, 3, 5, 8, and 12 h. Plasma gemcitabine concentrations were monitored on day 15 before treatment and 45, 60, 65, and 75 min and 1.5, 2, 2.5, 3, and 5 h after the administration of tipifarnib. On day 22, pharmacokinetic monitoring for tipifarnib alone was repeated at the same times as on day 15. For each sample, at least 5 ml of whole blood was collected in heparinized tubes, kept on ice, and centrifuged within 1 h to isolate plasma. Plasma was frozen immediately and stored at −20°C or less for later analysis.

Tipifarnib concentrations were measured in thawed plasma samples using a previously validated analytical method (17). Briefly, 1-ml aliquots of plasma were alkalized with 0.1 M NaOH, extracted with heptane-isoamyl alcohol (90:10, v/v), and analyzed using reverse-phase HPLC with UV detection at 240 nm. Samples were separated using a C18 BDS-Hypersil HPLC column (10 cm × 4.6 mm; particle size, 3 μ; Alltech, Deerfield, IL). The mobile phase consisted of 0.01 M ammonium acetate-acetonitrile (52:48, v/v). The lower limit of quantitation for tipifarnib was 2 ng/ml. The chromatographic peaks were quantified using UV detection at 240 nm. The mean overall coefficient of variation was 6.7% at 14.9 ng/ml, 7.1% at 124 ng/ml, and 7.1% at 2,064 ng/ml.

Gemcitabine plasma concentrations were analyzed using a HPLC-based assay (23). Briefly, acetic acid (2 ml, 1 μ) was added to 0.2-ml aliquots of patient plasma and mixed at 4°C. The aliquot was spiked with 200 ng of internal standard (2’,3’-dideoxyctydine) contained in 100 μl of methanol at room temperature. Thereafter, another 100 μl of methanol and 1 ml of 1 M acetic acid were added to the aliquot. The mixture was poured very slowly over a solid-phase extraction column (Oasis MCX solid phase extraction column, 3 ml/60 mg; Waters). The solid-phase extraction column was then washed with 3 ml of Milli-Q water, 1 ml of 1 M acetic acid, and 3 ml of methanol. Finally, gemcitabine was eluted with 3 ml of methanol-NH₄OH 25% (98:2, v/v). The aliquot was dried under nitrogen at 65°C. The extraction residue was redissolved in 200 μl of HPLC solvent mixture containing 0.01 M ammonium acetate (pH 9 with 25% NH₄OH) and methanol (90:10, v/v). The extractant (10 μl) was injected into a HPLC system that included a reverse-phase HPLC column (10 cm × 4.6 mm inner diameter) packed with 3 μm of C18 BDS-Hypersil (Alltech). The HPLC mobile phase initially consisted of 0.01 M ammonium acetate (pH 9 with 25% NH₄OH) and methanol (93.5:6.5, v/v), which was pumped isocratically at 0.8 ml/min for 5.6 min. The percentage of methanol was then increased to 70% from 6.5% at 5.6 min and finally reduced to 6.5% at 8.6 min after starting the HPLC run. The mobile phase, consisting of 0.01 M ammonium acetate and methanol (93.5:6.5, v/v), was held constant for 12 min. The overall HPLC run time was 12 min. With UV detection at 275 nm, the retention times of gemcitabine and 2’,3’-dideoxyctydine, the internal standard, were 4.2 and 5.6 min, respectively. The concentration of gemcitabine in patient plasma samples was calculated by determining the ratio of the area of
peak gemcitabine in each sample to that of the internal standard in each sample and comparing this ratio to a calibration curve obtained from known samples in the same HPLC running batch. The lower limit of quantitation for gemcitabine was 100 μg/ml.

**Farnesylation Assay.** To assess the serial effects of tipifarnib on the proportions of farnesylated and unfarnesylated forms of the chaperone protein HDJ2, which may reflect the effect of drug on protein farnesylation, PBMCs were obtained from approximately 8 ml of whole blood collected in sodium citrate vacuum tubes (Becton-Dickinson, Franklin Lakes, NJ) and processed within 10 min of collection. The PBMCs were stored at −70°C. The cells were homogenized on ice in 10 mM Tris (pH 7.4) and 0.3% SDS with 1% protease inhibitors (Sigma Chemical Co., St. Louis, MO) and centrifuged at 12,000 × g at 4°C. The supernatants were treated with 50 μg/ml DNase and 100 μg/ml RNase. The protein concentrations assessed by 260/280-nm absorbance ranged from 1–5 mg/ml (24). An equal amount of protein (20 μg) was boiled in SDS loading buffer containing 0.1 mM DTT and electrophoresed in 7.5% SDS polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA). Recombinant human HDJ2, generated by Drs. Matsatake Mori and Kazutoyo Terada (Kumamoto University, Kumamoto, Japan) or purchased from Neomarkers (Fremont, CA), were used as positive controls. The gels were transblotted to Hybond-C nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ), and the membranes were blocked in 0.1% Tween 20 and 10% SuperBlock (Pierce Chemical Co., Rockford, IL.). After incubation with either rabbit polyclonal anti-HD2 (generated by Drs. Mori and Terada) or monoclonal mouse anti-HD2 clone KA2A5.6 (Neomarkers), then goat anti-mouse (IgM) peroxidase conjugate (Calbiochem-Novabiochem Corporation, San Diego, CA) or goat antirabbit IgG (Abcam, Cambridge, United Kingdom), HDJ2 was visualized by enhanced chemiluminescence with SuperSignal West Pico reagent (Pierce Chemical Co.). Image analysis was performed with a personal densitometer (Amersham Biosciences). Relative quantities of farnesylated and unfarnesylated HDJ2, the latter detected as a slower migrating band, were determined using ImageQuant (Amersham Biosciences), and a percentage of unprenylated HDJ2 was calculated (25). Percentage (absolute) change in unfarnesylated HDJ2 was calculated by subtracting pretreatment values obtained on day 1 from day 8 or day 15 values. Comparisons were made between the day 1 and day 8 values where possible; however, if the day 8 sample was not available or could not be analyzed, then the day 15 value was used. Given that tipifarnib concentrations were expected to have achieved steady state by day 8, it was hypothesized that both days 8 and 15 would provide a reasonable index of the farnesylation status of HDJ2 after exposure to therapeutic levels of tipifarnib. Descriptive statistics were used to obtain the median percentage change in the proportion of unfarnesylated HDJ2 at the 100-, 200-, and 300-mg BID dose levels of tipifarnib. The percentage of unfarnesylated HDJ2 before treatment was compared with values on day 8 or day 15 at each dose level using a paired t test. The relationships between the percentage change in unfarnesylated HDJ2 protein and tipifarnib area under the concentration-time curve from zero to 12 h (AUC0–12 h) on days 15 and 22 were also explored.

Pharmacokinetic and Pharmacodynamic Analysis.
Gemcitabine and tipifarnib pharmacokinetics were examined using noncompartmental analytical methods with WinNonLin software (version 3.1; Pharsight Corp., Cary, NC). The AUC0–12 h was calculated using the linear trapezoidal method (26). The terminal rate constant (λz), was determined from the slope of the terminal log-linear portion of the plasma concentration-time curve, and the terminal half-life was calculated as ln (2)/λz. Maximal plasma concentrations (Cmax) were determined by direct observation of the data.

The relationships between indices of tipifarnib and gemcitabine exposure (Cmax and AUC) and myelosuppression in the first course of therapy were explored. In these pharmacodynamic analyses, myelosuppression was quantified as the percentage decrements in the ANC and platelet counts using the following formula:

\[
\text{Percentage decrement} = \frac{\text{Pretreatment blood cell counts} - \text{Nadir blood cell counts}}{\text{Pretreatment blood cell counts}} \times 100%
\]

Pharmacodynamic data were analyzed using regression methods to fit the data to linear, maximum effect, and sigmoidal maximum effect (Emax) models using WinNonLin software (version 3.1; Pharsight Corp.). Model selection and goodness-of-fit were assessed by examining the coefficients of determination (r²), graphical plots of the observed versus predicted data, and the SEs of the estimated parameters.

**Statistical Analysis.** The tipifarnib pharmacokinetic parameters on day 15 (in the presence of gemcitabine) and day 22 (in the absence of gemcitabine) were compared using an ANOVA test. Similarly, an ANOVA test was also used to compare the gemcitabine pharmacokinetic parameters on day 1 (in the absence tipifarnib) and day 15 (in the presence of tipifarnib). Thus, the clearance of each agent was assessed alone and together. A significance level of 0.05 was used for all
analyses. The results obtained from the ANOVA were used to generate 90% confidence intervals about the geometric mean ratios of the pharmacokinetic parameters with (test) and without (reference) interacting drugs. All statistical analyses were performed using the SAS statistical software program (version 6.21; SAS Institute, Inc., Cary, NC).

RESULTS

General. Twenty-two patients, whose pertinent characteristics are summarized in Table 1, were treated with 77 total courses of tipifarnib/gemcitabine, with 19 patients and 74 courses fully evaluable for toxicity assessment. Twelve patients had previously received chemotherapy and/or radiation therapy. The total numbers of new patients and fully evaluable courses, at each tipifarnib dose level, as well as the rates of DLT as a function of dose level, are detailed in Table 2.

In summary, tipifarnib dose escalation proceeded in the following manner. Three new patients tolerated tipifarnib/gemcitabine (mg BID) at the first dose level of 100/1000 without DLT, and, thus, dose level escalation proceeded to 200/1000, which was also well tolerated in the first three patients. However, the next higher dose level, 300/1000, resulted in an acceptably high rate of hematological DLT. Therefore, additional patients were treated at the preceding dose level of 200/1000, and this resulted in dose-limiting hematological toxicity in 2 of 11 fully evaluable new patients. Therefore, the recommended Phase II dose level was 200 mg of tipifarnib BID in combination with 1000 mg/m² gemcitabine i.v. on days 1, 8, and 15 every 28 days. When reviewing all dose levels, 9 of 19 evaluable patients required omission and/or reduction in the dose of gemcitabine at some time during treatment. Gemcitabine dose reductions occurred in 19% of all courses (15% because of hematological toxicity and 4% because of nonhematologic toxicity), with 13% and 6% occurring at the second and third dose levels, respectively.

Hematological Toxicity. Myelosuppression, particularly neutropenia, was the principal toxicity of the tipifarnib/gemcitabine combination. The median, range, and distribution according to National Cancer Institute grade of the nadir ANC and platelets as functions of both dose level and the extent of prior therapy are shown in Table 3. The ANC nadir during the first course typically occurred between days 15 and 22, with neutropenia resolving within 7 days. Neutropenia of grade 3 or grade 4 severity occurred in 13 (18%) of 74 courses and was dose limiting in 3 (4%) courses. Thrombocytopenia of grade 3 severity was observed in 4 (5%) of 74 courses, with one episode being dose limiting at 200 mg of tipifarnib BID. The dosage of tipifarnib was required to be reduced for prolonged grade 4 neutropenia in only one patient (300/1000 dose level). The first dose level (100/1000) was associated with brief grade 3 neutropenia in 1 (1%) of 15 evaluable courses. A total of 48 courses were administered at the second dose level (200/1000), of which seven (9%) were associated with grade 3 neutropenia, two (3%) with brief grade 4 neutropenia, one (1%) with grade 4 neutropenia with fever, and three (4%) with grade 3 thrombocytopenia. Unacceptable grade 4 neutropenia lasting more than 5 days was observed in 2 (3%) of 11 courses at the 300/1000 dose level, and, in one of these, grade 3 thrombocytopenia lasting 13 days was also observed.

Nonhematological Toxicity. Nonhematological toxicities were mild to moderate in severity in most patients. Grade 1 and/or grade 2 nausea or vomiting occurred in 28 (38%) of 74 and 16 (22%) of 74 courses, respectively, and was typically managed with oral phenothiazines. Fatigue of grade 1 or grade 2 severity was observed in 37 (50%) of 74 courses, whereas isolated grade 3 fatigue was observed in a single patient during a first course at the 200/1000 dose level. The latter event, which was brief and experienced after the first gemcitabine treatment, did not recur with subsequent treatments. Given its transient, nonreproducible nature and the lack of distinct association with the tipifarnib/gemcitabine regimen, the event was not classified as dose limiting. Mild to moderate anorexia occurred in 11 (15%) of 74 courses, whereas grade 3 anorexia was observed in a single patient at the 300/1000 dose level in association with dose-limiting hematological toxicity. Mild to moderate diarrhea, fever, and skin rash were observed in <20% of courses. One instance of grade 3 rash was observed at the 200/1000 dose level in association with dose-limiting hematological toxicity. Asymptomatic grade 1 or grade 2 hepatic transaminase elevations were observed in 16 (22%) of 74 courses and were thought to be caused by gemcitabine. A single patient at the second dose level developed a moderate (grade 2) sensory neuropathy after six courses of treatment. The pertinent past medical history of this patient, a 52-year-old male with previously untreated metastatic pancreatic cancer, included extensive tobacco use, hyperlipidemia, and noninsulin-dependent diabetes mellitus. Tipifarnib treatment was omitted during the eighth course, but the patient continued to receive gemcitabine. The neuropathy improved to grade 1 level, and, thus, tipifarnib was restarted at a dose of 200 mg BID for the ninth course. The recurrence of grade 2 sensory neuropathic symptoms ultimately resulted in dose reduction of tipifarnib to 150 mg BID for two additional courses.
courses, in which partial resolution of symptoms to grade 1 severity was observed.

**Antineoplastic Activity.** Two partial responses were observed. A 73-year-old male with previously untreated metastatic pancreatic cancer (100/1000 dose level) experienced a partial response lasting 6 months. The second partial response (300/1000 dose level), which lasted for 5 months, was observed in a 34-year-old patient with metastatic nasopharyngeal carcinoma who had received prior chemotherapy and radiation. Furthermore, two previously untreated patients with metastatic pancreatic cancer experienced 2.5- to 15-fold reductions in serum CA 19-9, as well as improvement in pain and performance status for 7–9 months.

**Pharmacokinetics.** Plasma samples for pharmacokinetic studies were obtained from 15 and 12 patients for tipifarnib and gemcitabine, respectively. Fig. 2 shows the mean steady-state plasma tipifarnib concentration-time profiles at the 200-mg BID dose level in the presence and absence of gemcitabine. Fig. 3 shows paired tipifarnib AUC<sub>0-12</sub> values for individual patients in the presence and absence of gemcitabine, and tipifarnib pharmacokinetic parameters in the presence and absence of gemcitabine are shown in Table 4. There were no significant differences in pharmacokinetic parameters reflecting tipifarnib exposure when the agent was administered alone or in the presence of gemcitabine. As shown in Table 5, which displays plasma tipifarnib and gemcitabine pharmacokinetic parameters for each drug administered alone and in combination, there was no major effect of gemcitabine on the clearance of tipifarnib across all three tipifarnib dose levels.

Mean plasma gemcitabine concentration-time profiles after treatment with gemcitabine in the presence and absence of tipifarnib and paired gemcitabine AUC<sub>last</sub> (area under concentration-time curve from time zero until the last measured time point) values for individual patients in the presence and absence of tipifarnib are shown in Figs. 4 and 5, respectively. As shown in these figures, there were no significant effects of tipifarnib on mean plasma gemcitabine concentrations and AUC<sub>last</sub>, which

<table>
<thead>
<tr>
<th>Tipifarnib dose level (mg BID)</th>
<th>No. of evaluable courses</th>
<th>No. of courses (first courses) with toxicity</th>
<th>Median nadir ANC (range; /H9262 l)</th>
<th>Platelets (range; /H9262 l)</th>
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<tbody>
<tr>
<td>100</td>
<td>15</td>
<td>3009 (105–10,722)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>200</td>
<td>48</td>
<td>3082 (45–7,690)</td>
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<tr>
<td>300</td>
<td>11</td>
<td>2200 (72–22,649)</td>
<td>0 (0)</td>
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> Hematologic toxicity.

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**Table 3**

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<tr>
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<th>No. of evaluable courses</th>
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> Hematologic toxicity.

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**Fig. 2** Mean (±SD) steady-state plasma tipifarnib concentration-time profiles after treatment with 200 mg of tipifarnib BID with (day 15) and without (day 22) concomitant administration of gemcitabine (1000 mg/m²; days 1, 8, 15, every 28 days; n = 8).
reflect drug exposure. This is further illustrated in Tables 5 and 6, which compare pharmacokinetic parameters of gemcitabine in the presence and absence of tipifarnib and fail to demonstrate major differences across the dose levels of tipifarnib evaluated in this study (100–300 mg BID). The results indicate that there are no major interactions of gemcitabine on tipifarnib clearance and exposure, and vice versa.

**Pharmacodynamics.** Pharmacodynamic relationships between hematological effects and pertinent pharmacokinetic parameters reflecting both tipifarnib and gemcitabine exposure were explored. No significant relationship was demonstrated between tipifarnib AUC_{0–12 h} and the percentage decrements in platelet counts on day 15 (presence of gemcitabine) and day 22 (absence of gemcitabine) using linear and E_{max} models. Similarly, the relationship between tipifarnib AUC_{0–12 h} and the percentage decrements in ANC on days 15 and 22 were described by neither linear nor E_{max} models. No pharmacodynamic relationships between gemcitabine exposure and the percentage decrement in platelet or ANC were evident.

**Farnesylation Studies.** Blood was serially sampled in 16 patients to assess the effects of the tipifarnib/gemcitabine regimen on farnesylation of the chaperone protein HDJ2. In course 1, the proportion of unfarnesylated HDJ2 increased in 12 (75%) of 16 patients. The median percentage changes in the proportion

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*Fig. 3* Paired tipifarnib AUC_{0–12 h} values for individual patients in the presence (day 15) and absence (day 22) of gemcitabine.

### Table 4  Summary of mean tipifarnib plasma pharmacokinetic parameters in the presence and absence of gemcitabine

<table>
<thead>
<tr>
<th>Pharmacokinetic variable</th>
<th>Units</th>
<th>Tipifarnib alone&lt;sup&gt;a&lt;/sup&gt; (day 22)</th>
<th>Tipifarnib and gemcitabine&lt;sup&gt;c&lt;/sup&gt; (day 15)</th>
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<tr>
<td>Tipifarnib (100 mg BID; n = 4)</td>
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<td></td>
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</tr>
<tr>
<td>AUC&lt;sub&gt;0–12 h&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ng/h/ml</td>
<td>1985 (832)</td>
<td>2452 (648)</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
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<tr>
<td>AUC&lt;sub&gt;0–12 h&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ng/h/ml</td>
<td>2552 (1294)</td>
<td>2171 (1229)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>ng/ml</td>
<td>525 (260)</td>
<td>468 (355)</td>
</tr>
<tr>
<td>Tipifarnib (300 mg BID; n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–12 h&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ng/h/ml</td>
<td>2833 (1387)</td>
<td>3369 (1911)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>ng/ml</td>
<td>536 (260)</td>
<td>805 (490)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values represent mean (SD).

<sup>b</sup>AUC<sub>0–12 h</sub> area under the time-concentration curve from zero to 12 h; C<sub>max</sub>, maximum plasma concentration.

### Table 5  Comparison of tipifarnib and gemcitabine pharmacokinetic parameters for each agent administered alone and in combination

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Tipifarnib pharmacokinetic parameters (100–300 mg BID, n = 15) with or without gemcitabine (1000 mg/m²)</th>
<th>Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>90% confidence interval&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;0–12 h&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ng/h/ml</td>
<td>0.932</td>
<td>99.3</td>
<td>(86.5; 114.0)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>ng/ml</td>
<td>0.999</td>
<td>100.0</td>
<td>(79.4; 125.9)</td>
</tr>
<tr>
<td>Gemcitabine pharmacokinetic parameters (1000 mg/m², n = 12) with or without tipifarnib (100–300 mg BID)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;last&lt;/sub&gt;</td>
<td>ng/h/ml</td>
<td>0.898</td>
<td>99.7</td>
<td>(67.7; 146.8)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>ng/ml</td>
<td>0.640</td>
<td>109.2</td>
<td>(78.6; 151)</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>min</td>
<td>0.201</td>
<td>73.5</td>
<td>(38.5; 108.5)</td>
</tr>
</tbody>
</table>

<sup>a</sup>P (two-sided) in log scale for testing a zero difference between two treatments.

<sup>b</sup>Based on least square means; calculated as the ratio of test (tipifarnib and gemcitabine) to reference (tipifarnib or gemcitabine alone).

<sup>c</sup>Confidence limits in log scale are expressed in percentages of tipifarnib alone.

<sup>d</sup>AUC<sub>0–12 h</sub> area under the time-concentration curve from zero to 12 hours; C<sub>max</sub>, maximum plasma concentration; AUC<sub>last</sub> area under the time-concentration curve from zero to the last measurable time point; T<sub>1/2</sub>, elimination half-life.
Phase I Trial of Tipifarnib and Gemcitabine

In preclinical studies with tipifarnib, cells harboring mutated ras, and prominent activity in early clinical trials in breast, central nervous system, and other malignancies that have low incidences of ras mutations suggest that the FTIs may broadly affect the farnesylation of many proteins in addition to Ras.

Given the current ambiguity surrounding the mechanism by which the FTIs exert antitumor activity, this Phase I, pharmacological and biological study was designed to determine the MTDs and overall feasibility of administering the nonpeptidomimetic tipifarnib in combination with gemcitabine, as well as to identify major pharmacokinetic interactions between the agents and evidence of inhibition of HDJ2 chaperone protein farnesylation. The rationale for evaluating this particular regimen was largely based on the high incidence of ras mutations in carcinomas of the pancreas and lung and other malignancies in which gemcitabine is a mainstay of treatment, particularly because the role of a tipifarnib/gemcitabine regimen that is feasible from both toxicological and pharmacological perspectives could be further evaluated in pivotal first-line clinical trials. Indeed, the results of the present study indicate that the combined administration of biologically and clinically relevant doses of both tipifarnib and gemcitabine is feasible. The principal toxicities of the tipifarnib/gemcitabine combination were neutropenia, with neutropenia of grade 3–4 severity occurring in 18% of all courses. The incidence of grade 3 thrombocytopenia was even lower, occurring in 5% of all courses, whereas grade 4 thrombocytopenia was not observed. At the highest dose level of tipifarnib/gemcitabine (300/1000) evaluated in the study, the incidence of intolerable myelosuppression was acceptably high, occurring in two of five patients in first courses. In contrast, the next lower tipifarnib/gemcitabine dose level (200/1000) was better tolerated. At this dose level, which was considered the MTD level and recommended for subsequent disease-directed studies, dose-limiting hematological events were much less frequent, occurring in 2 of 11 (11%) evaluable patients. Furthermore, few patients treated at this dose level required either dose modification, reduction, or omission, even after cumulative, long-term treatment. In fact, gemcitabine doses were reduced at some time during treatment because of insufficient blood cell counts on the day of treatment in only 11% of 74 total courses in the study. In addition, both first-course and cumulative nonhematological toxicities were generally mild to moderate at the MTD level. Although a single individual developed a grade 2 peripheral neuropathy, which has been reported in other clinical evaluations of tipifarnib administered as both a single agent and in various combination regimens, manifestations of neurotoxicity were of maximal severity after repetitive drug administration and the patient had several major risk factors for developing peripheral neuropathy (17, 19, 20).

Because the pharmacokinetic behaviors of both tipifarnib and gemcitabine have been extensively characterized in previous clinical investigations, the principal objective of the pharmacokinetic assessments in the present trials was to determine the propensity for major pharmacokinetic interactions with a principal focus on drug clearance. Congruent with the lack of major toxicological interactions, which is supported by the feasibility of administering both agents together at full single-agent doses, there was no evidence for major pharmacological interactions between tipifarnib and gemcitabine. Although the results of the present study do not refute the possibility of pharmacokinetic interactions of a lesser magnitude, the existence of functionally relevant effects of tipifarnib on the clearance of gemcitabine, and visa versa, is improbable. Furthermore, the range of tipifarnib plasma concentrations at the recommended dose level of 200 mg of tipifarnib BID in combination with 1000 mg/m² gemcitabine on days 1, 8, and 15 every 28 days (minimum plasma concentration, 14–305 ng/ml) is similar to that noted in single-agent studies and exceeds tipifarnib concentrations capable of inhibiting farnesylation of Ras and other critical proteins, and, importantly, tumor growth in preclinical studies (6, 14). In preclinical studies with tipifarnib, cell lines bearing H-ras or N-ras mutations demonstrated IC₅₀ for inhibition of proliferation below 5 ng/ml (14).

The effects of the combination of tipifarnib and gemcitabine on the farnesylation of HDJ2, a chaperone protein that undergoes farnesylation, was also assessed in the present study.
The proportion of unfarnesylated HDJ2 protein increased in 12 of the 16 patients who had serial sampling performed; however, these results and similar studies assessing the farnesylation status of other readily quantifiable proteins, such as lamin A, must be interpreted with caution for several reasons. First, although the inhibition of HDJ2 farnesylation may indicate that tipifarnib is truly affecting its intended target, FTase, which is in line with its purported biochemical mechanism, the degree of concordance between the farnesylation status of the HDJ2 chaperone protein, Ras, and/or other farnesylated proteins that are linked to clinical end points is not known. Therefore, serial evaluations of HDJ2 farnesylation may be used as general pharmacodynamic indices of protein farnesylation, however, concordant studies relating the farnesylation of HDJ2 to that of proteins linked to tumor proliferation are necessary to determine the overall use and significance of such assessments.
ingly, the results of preclinical and clinical studies of the effects of L-778,123, an inhibitor of FTase, as well as GGTase I, to a lesser degree, on various prenylated proteins clearly demonstrated that the agent was highly capable of inhibiting HDJ2 farnesylation, whereas the prenylation of K-Ras was not affected (27). Although these results are likely explained by the alternate prenylation of N- and K-Ras by residual GGTase I, they illustrate the lack of correlation between the inhibition of FTase activity and Ras functionality. Another factor that may potentially confound the interpretation of the HDJ2 farnesylation data in this and other clinical trials, to date, is the lack of robust information relating drug effects on farnesylation in PBMCs to tumor tissues in humans. A final issue pertaining to assessing the farnesylation status of HDJ2 and other proteins that might suffice as surrogates of relevant target effects is not knowing the precise mechanism by which FTIs inhibit tumor proliferation and/or induce cytotoxicity. This is shown by the responsiveness of cancers with both wild-type ras and K-ras mutations in preclinical studies and clear tumor regressions in patients with advanced malignancies with an inherently low incidence of ras mutations including breast carcinoma, high-grade astrocytoma, and several types of hematopoietic malignancies (20, 21, 28, 29). Although the activity of the FTIs in these situations may be accounted for by inhibition of Ras farnesylat and hyperactive signaling activity upstream of Ras, there is a large body of preclinical evidence implicating RhoB, Akt2, centromere-associated protein-E, centromere-associated protein-F, and other farnesylated proteins as the principal target of the FTIs (10–13). The possibility that the principal target of the FTIs is tumor dependent presents additional challenges to identifying and developing feasible and reliable assays that reflect the effects of the FTIs on tumor growth.

The present study demonstrated the administration of the combination of 200 mg of tipifarnib BID plus 1000 mg/m² gemcitabine i.v. weekly for 3 weeks every 28 days is feasible, and these doses are recommended for further disease-directed studies of the regimen. At these doses, major drug-drug interactions were not evident, and farnesylation of HDJ2 in PBMCs was inhibited in most patients in whom serial assessments were performed. Nevertheless, treatment with the tipifarnib/gemcitabine regimen at these doses should be limited to patients with good performance status, organ function, and a history of no more than minimal myelotoxic therapy similar to those in the present study until patients who are potentially at higher risk for toxicity are studied. The tolerance of this regimen has been further substantiated in a Phase III study, in which 688 patients with advanced pancreatic cancer were randomized to treatment with either the identical tipifarnib/gemcitabine regimen or gemcitabine on an identical dose schedule plus placebo (22). The survival of patients treated in the experimental arm was similar to that of patients treated with gemcitabine alone. The results of this Phase III study alone should not preclude further evaluations of the tipifarnib/gemcitabine regimen in other malignancies, such as metastatic breast cancer, in which the single-agents tipifarnib and gemcitabine each have reported activity (20, 30, 31). It is of interest that the development of tipifarnib continues in hematological malignancies, gliomas, and breast cancer (28, 29).

REFERENCES


