A Phase I and Pharmacological Study of the Farnesyl Protein Transferase Inhibitor L-778,123 in Patients with Solid Malignancies

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ABSTRACT

This Phase I study was performed to assess the feasibility of administering L-778,123, a peptidomimetic farnesyl protein transferase (FPTase) inhibitor, as a continuous i.v. infusion for 7 days every 3 weeks and to determine the recommended dose for subsequent disease-directed trials. This study also sought to characterize the pharmacological behavior of L-778,123 and to determine whether the desired biological effect, inhibition of protein farnesylation, could be detected and assessed during treatment. Patients with advanced solid malignancies were treated with L-778,123 as a continuous i.v. infusion for 7 days every 3 weeks at doses ranging from 35 to 1120 mg/m²/day. On the basis of preclinical studies, toxicity assessments included cardiac telemetry, electrocardiograms, and electroretinograms in addition to more routine safety monitoring laboratory tests. Plasma sampling was performed to characterize the pharmacokinetics of L-778,123, and peripheral blood mononuclear cells (PBMCs) were sampled to detect and monitor the inhibitory effects of L-778,123 on the prenylation of HDJ2, a chaperone protein that undergoes farnesylation. Twenty-five patients received 51 complete courses of L-778,123. An unacceptably high incidence of dose-limiting toxicities, consisting of grade 4 thrombocytopenia, significant prolongation of the QT interval, and profound fatigue, was observed at the 1120 mg/m²/day dose level. At the next lower L-778,123 dose level, 560 mg/m²/day, seven new patients had no unacceptable toxicity. Instead, myelosuppression was mild to moderate and QT prolongation was negligible. Pharmacokinetics were linear, and L-778,123 plasma concentrations at steady-state (mean, 8.09 ± 3.11 μM at 560 mg/m²/day) exceeded IC₅₀ values (range, 0.07–5.35 μM) required for growth inhibition and cytotoxicity in preclinical studies. The systemic clearance of L-778,123 averaged 106.4 ± 45.6 ml/min/m², and the terminal half-life of elimination was 2.8 ± 1.0 h. L-778,123 inhibited HDJ2 prenylation for the duration of the drug infusion in a dose-dependent manner, but seemed to plateau above 560 mg/m²/day. At the 560 mg/m²/day dose level, the mean percentage of HDJ2 protein in its unprenylated form increased from 1.41% ± 1.71% (pretreatment) to 28.76% ± 6.10% (day 4) and 30.86 ± 4.96 (day 8) and declined to 2.28% ± 2.11% one week after drug discontinuation (day 16). L-778,123 administered as a continuous 7-day i.v. infusion for 7 days every 21 days is well tolerated at doses of 560 mg/m²/day and results in biologically relevant concentrations and consistent inhibition of HDJ2 prenylation in PBMCs. Although the relationship between drug-related inhibition of HDJ2 prenylation in PBMCs and both prenylation of relevant proteins and growth inhibition in tumor cells is unknown, serial analyses of HDJ2 prenylation provide a pharmacodynamic marker of protein prenylation that may be useful in optimizing the development of drugs targeting FPTase.

INTRODUCTION

FPTase modifies proteins terminating in the CAAX motif, a specific COOH-terminal tetrapeptide sequence consisting of a cysteine followed by two aliphatic amino acids and a terminal serine or methionine (1–3). FPTase catalyzes the covalent transfer of a 15-carbon isoprenyl moiety from the cholesterol intermediate farnesyl PP, to the CAAX cysteine residue, thereby rendering the protein substrate more hydrophobic (1–3). Two other structurally related protein prenyl transferases, GGPTases I and II, prenylate critical proteins by attaching either one or two 20-carbon geranylgeranyl lipid moieties to the COOH-terminal end of proteins (1–3). Prenylation is critical not only for the localization of proteins involved in diverse physiological processes such as skeletal muscle function and vision, but also for...
the localization of proteins that are oncogenic and/or have roles in mitogenic signaling (1–3).

FPTase was first identified as a target for drug development when it was discovered that farnesylation is the critical step in promoting the transforming potential of mutated ras (1–6). Under normal conditions, farnesylated H-Ras, K-Ras, and N-Ras associate with the inner plasma membrane, where they are triggered by various growth factors and cytokines to activate a series of downstream effectors capable of inducing cell growth and proliferation (7–9). In approximately 30% of human cancers, however, mutations in ras oncogenes produce constitutively activated Ras, leading to the continuous propagation of a proliferative signal (1, 7, 10). FPTase inhibitors were initially developed to inhibit signaling pathways emanating from mutated Ras by preventing the localization of Ras to the inner plasma membrane (1–3). Several lines of evidence, however, have indicated that Ras may not be the principal target of this class of compounds, including the fact that ras mutation status does not predict for FPTase cytotoxicity (1–3, 11–13). Other proposed targets of FPTase inhibitors include farnesylated proteins such as lamins A and B, RhoB, or CNEP-E and -F (11–15), although the mechanism by which FPTase inhibitors manifest their antitumor activity remains uncertain.

L-778,123 (Fig. 1) is a membrane-permeable peptidomimetic FPTase inhibitor. This CAAX analogue competes with protein substrates for FPTase, with an inhibition constant (Kᵢ) ranging from 0.3 ± 0.2 nM for N-Ras to 1.6 ± 1.3 nM for H-Ras. L-778,123 inhibits the anchorage-independent growth of Rat-1 rodent fibroblasts transformed by both ras and raf (16). This compound is active against human cancer cell lines harboring both mutated K-ras and wild-type ras, with IC₅₀ values of 2280–5350 nM and 70–547 nM, respectively (16). In preclinical toxicity studies in rodents and dogs, myelosuppression and prolongation of the QTₑ interval were among the principal adverse effects (16). Dogs receiving 24-h continuous i.v. infusions experienced transient prolongations of the QTₑ interval by 10–15%, with subsequent normalization after discontinuation of L-778,123 (16). In addition, ocular effects, consisting of electroretinographic changes, were observed in one dog receiving a continuous i.v. infusion at 50 mg/kg/day for 7 days (16), which may be related to drug effects on the farnesylation of several essential retinal signaling proteins (1–3).

On the basis of the novel mechanism of action of L-778,123 as an FPTase inhibitor, as well as the agent’s favorable antitumor activity and toxicity profiles in preclinical studies, L-778,123 was selected to undergo clinical development.

The principal objectives of this Phase I and pharmacological study were to: (a) describe the toxicities of L-778,123 administered as a continuous i.v. infusion for 7 days every 3 weeks in patients with advanced solid malignancies; (b) determine the MTD and recommended dose for subsequent clinical trials; (c) assess the pharmacological behavior of L-778,123; (d) seek preliminary evidence of antitumor activity in patients with advanced cancers; and (e) determine whether the inhibition of protein prenylation, specifically that of the HDJ2 chaperone protein, could be detected in the PBMCs of patients during treatment and its behavior as a function of L-778,123 dose, concentration, and treatment duration.

PATIENTS AND METHODS

Patient Selection. Patients with histologically confirmed advanced solid malignancies refractory to standard therapy or for whom no effective therapy existed were candidates for this study. Other eligibility criteria included: (a) age ≥18 years; (b) Eastern Cooperative Oncology Group performance status of 0 to 1 (ambulatory and capable of light work); (c) life expectancy of at least 12 weeks; (d) presence of measurable or evaluable disease; (e) no known untreated brain metastases or history of a seizure disorder; (f) no chemotherapy, radiotherapy, or investigational agents in the previous 4 weeks; (g) no nitrosoareas or mitomycin C within the previous 6 weeks; (h) no prior high-dose chemotherapy with stem cell rescue; (i) cumulative anthracycline dose not exceeding the equivalent of 450 mg/m² of doxorubicin; (j) no myocardial infarction, unstable angina, or congestive heart failure within the past 12 months; (k) adequate hematopoietic (absolute neutrophil count ≥1,500/µL, platelet count ≥100,000/µL, and hemoglobin ≥9.0 g/dl), hepatic (total serum bilirubin ≤1.5 times the upper limit of normal, transaminases ≤2.5 times the upper limit of normal, and alkaline phosphatase ≤4 times the upper limit of normal), and renal (serum creatinine concentration ≤1.5 times the upper limit of normal) functions; (l) prothrombin and partial thromboplastin times the upper limit of normal; (m) serum potassium, calcium, and magnesium within the normal range and other serum electrolytes within 10% of the normal range; (n) no history of significant cardiac dysrhythmias (atrial fibrillation or grade 3 dysrhythmia) or QTₑ abnormalities; (o) QTₑ <440 ms on screening ECG; (p) no concomitant use of medications with dysrhythmic potential (including, but not limited to, terfenadine, astemizole, cisapride, diphenhydramine, quinidine, procainamide, disopyramide, sotalol, probucol, bepridil, tricyclic antidepressants, haloperidol, risperidone, and indapamide); (q) no concomitant use of medications which induce CYP3A (including, but not limited to, rifampin, phenobarbital, phenytoin, carbamazepine, troglitazone, and rifabutin, because L-778,123 is primarily metabolized by CYP3A4), or CYP3A-metabolized benzodiazepines, or 3-hydroxy-3-methylglutaryl acetyl-CoA reductase inhibitors; (r) no history of a significant retinal disorder or disease; and (s) no other coexisting medical problems of sufficient severity to prevent full compliance with the study. Females of childbearing age were required to be practicing effective contraceptive measures and to have had a negative serum pregnancy test before study entry. Written informed

![Fig. 1 Chemical structure of L-778,123.](image-url)
Drug Dosage and Escalation. The starting dose of L-778,123 was 35 mg/m²/day administered as a continuous i.v. infusion over 24 h daily for 7 days every 3 weeks. This starting dose was predicted to produce plasma L-778,123 concentrations of ∼0.25–0.5 μM, which were associated with therapeutic activity in murine studies. This starting dose was equivalent to one-twelfth of the toxic dose low in dogs and less than one-tenth of the dose that resulted in lethality in 10% of mice. L-778,123 doses were to be doubled in each successive group of new patients until one patient experienced drug-related grade 2 toxicity or significant QTc prolongation. Adverse experiences of nausea, vomiting, fatigue, anorexia, anemia, alopecia, fever, and local reactions were not considered in altering the increments used for dose escalation. After the occurrence of a non-QTc toxicity that was at least grade 2 in severity, subsequent dose escalation increments were selected according to a modified Fibonacci scheme. After the occurrence of significant QTc prolongation, subsequent dose escalations were not to exceed increments of 33%. It was planned to enroll at least three patients at each dose level.

DLT was defined as any one of the following: (a) grade 4 hematological toxicity, consisting of either absolute neutrophil count <500/μL, platelet count <25,000/μL, or hemoglobin <6.5 g/dL; (b) grade 3 hematological toxicity of >1-week duration; (c) irreversible grade 2 toxicity; and (d) QTc prolongation >490 ms, or an absolute increase in the QTc interval of >80 ms from the QTc interval documented pretreatment. If one episode of DLT occurred, a maximum of six patients were treated at that dose level. The MTD was defined as the lowest dose in which at least two of six new patients experienced DLT, and the recommended Phase II dose was defined as the highest dose in which fewer than two of six new patients experienced DLT. Toxicities were graded according to the National Cancer Institute common toxicity criteria, version 1 (17).

Patients were permitted to continue treatment with L-778,123 at the same dose level as long as there was no evidence of progressive disease or ongoing drug-related toxicity at the time of reassessment and as long as they had not experienced: (a) prolongation of the QTc interval to ≥440 ms; (b) grade ≥2 cardiac dysfunction; (c) grade ≥1 neurotoxicity or visual disturbance; (d) grade ≥4 myelosuppression; or (e) other toxicity which was greater than grade 3 in severity. Those patients who developed prolongation of the QTc interval to between 440 and 489 ms were eligible to continue treatment at 50% of their initial dose, but those patients who developed prolongation of the QTc interval to ≥490 ms were not retreated. Likewise, patients who experienced either grade 2 cardiac dysfunction, grade 1 neurotoxicity, or grade 1 visual disturbance (including reproducible electroretinogram abnormalities) were eligible to receive treatment with L-778,123 at 50% of their initial dose, but those patients who developed more severe cardiac dysfunction, neurotoxicity, or visual disturbances could not receive additional treatment with L-778,123. Patients who experienced grade 4 hematological toxicity received a 25% dose reduction for treatment in subsequent courses. Regarding other toxicities, patients who had experienced grade 3 toxicities were permitted to continue treatment with L-778,123 at 25% of their initial dose, and patients who had experienced grade 4 toxicities were permitted to continue treatment at 50% of the initial dose.

Drug Administration. L-778,123 was provided by Merck Research Laboratories (West Point, PA) as a 10 or 50 mg/ml solution for i.v. infusion, and it was stored at 2 to 8°C. L-778,123 was diluted with normal saline to fill reservoirs of 100 to 1000 ml, depending on the dose level, and administered as a 7-day continuous i.v. infusion with a Deltec CADD-PLUS infusion pump (SIMS Deltec, Inc., St. Paul, MN) at a minimum infusion rate of 3.8 ml/h. Because diluted solutions of L-778,123 are stable for 1 week at 25°C, a maximum supply of 3 days was prepared at once.

Pretreatment and Follow-up Studies. Before each course of treatment, histories and physical examinations (with complete neurological examinations, funduscopic examinations, and visual acuity assessments) were performed by the oncologist, and the following evaluations were also obtained: a bilateral electroretinogram, 12-lead ECG, complete blood counts, routine chemistries and electrolytes, clotting studies, urinalysis, and relevant tumor markers. Before the first course of treatment only, a 24-h continuous ECG (Holter) recording was also obtained.

Intensive monitoring was performed during the first two courses of treatment, including continuous ECG monitoring for the first 24 h of the L-778,123 infusion. To serially monitor the QTc interval, 12-lead ECGs were obtained pretreatment, at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 48, 72, 96, 120, and 144 h after the start of the infusion, and on days 10, 14, and 17. On days 4, 8, and 17, physical examinations (with complete neurological examinations, funduscopic examinations, and visual acuity assessments), complete blood counts, routine chemistries, electrolytes, clotting studies, urinalyses were obtained. On day 8, a bilateral electroretinogram was also performed.

During subsequent courses, the monitoring of patients was less intensive. On days 1 and 4, physical examinations (with complete neurological examinations, funduscopic examinations, and visual acuity assessments), complete blood counts, routine chemistries, electrolytes, clotting studies, and urinalyses were obtained. Although continuous ECG monitoring was not required, a 12-lead ECG was obtained pretreatment, at 1, 3, and 72 h after the start of the infusion and on day 8.

Appropriate radiological studies for documentation of disease status were performed pretreatment and after every 2 courses. A complete response was defined as the disappearance of all known disease on two measurements, separated by a minimum of 4 weeks. A partial response required at least a 50% reduction in the sum of the products of the bidimensional measurements, separated by at least 4 weeks. Progressive disease was defined as an increase in the sum of the bidimensional products of all known disease by at least 25% or the appearance of new lesions.

Pharmacokinetic Sampling and Assay. To determine the pharmacokinetic behavior of L-778,123, blood samples were drawn during the first course of treatment. Blood was sampled immediately before the infusion, at 0.5, 1, 3, 12, 24, 48, 72, 96, 120, and 144 h during the infusion, immediately after discontinuation of the infusion on day 8, at 0.25, 0.5, 0.75, 1, 2, 4, 8, and 12 h after discontinuation of the infusion on day 8, and on days 10, 14, and 17. Five-ml samples were collected in tubes.
containing sodium heparin, placed on wet ice for 20 min, and then centrifuged at 2200 \times g for 10 min. A 2.5-ml plasma fraction was transferred to a 3.6-ml polypropylene tube and frozen at -20°C.

L-778,123 concentrations were measured by liquid chromatography and tandem mass spectrometry. L-778,123 and the internal standard, L-794,883, which differs from L-778,123 in having a propyl group on the imidazole ring, were isolated from 1 ml of plasma by automated solid-phase extraction on Isolute CN cartridges (International Sorbent Technology, Hengoed, South Wales) using a Gilson Aspec XL automated sample preparation system (Gilson Company, Inc., Lewis Center, OH). Reverse-phase chromatographic separation of L-778,123 from L-794,883 was achieved using a BDS Hypersil C8 high-performance liquid chromatography column (15 cm; Keystone Scientific, Inc., Bellefonte, PA) and a mobile phase composed of acetoniitrile, methanol, and water (50/44/6 by volume) containing 0.05% trifluoroacetic acid. Mass detection was accomplished using atmospheric pressure chemical ionization (heated nebulizer) and multiple reaction monitoring in the positive ion mode. Fragments of the parent ions, \textit{m/z} 406 \rightarrow 195 for L-778,123 and \textit{m/z} 448 \rightarrow 195 for L-794,883, were monitored using a 250-ms dwell time. Weighted (1/x²) linear regression analysis of peak area ratios of L-778,123:L-794,883 was used to generate data for calibration curves and to calculate the sample results.

The plasma calibration curve was linear over the concentration range of 0.5–1000 ng/ml. Mean accuracy for plasma standards (n = 6) ranged from 95.4 to 114.4%, with intraday coefficients of variation from 1.8 to 7.8%. Interday coefficients of variation for three sets of plasma quality control standards prepared at different times and assayed over the course of at least 2 months were all \pm 7.2%. The recovery of L-778,123 from replicate extracted plasma standards averaged 88.6 \pm 6.6%. The recovery of the internal standard, L-794,883, was similar to that of L-778,123. Freeze-thaw stability of L-778,123 in plasma stored at -20°C was demonstrated for three different concentrations through three freeze-thaw cycles over at least 3 months. Mean (n = 6) interday assays of plasma freeze-thaw samples ranged from 94.5 to 105.9% of nominal, with a coefficient of variation of \pm 8.1%. Assay specificity was evaluated using five different lots of human control plasma as well as plasma samples from patients enrolled in this study. L-778,123 and L-794,883 were chromatographically separated from endogenous peaks.

**Pharmacokinetic Analysis.** Individual L-778,123 plasma concentration-time data sets were analyzed by model-independent methods using the software program WinNonlin, version 1.1 (Statistical Consultants, Apex, NC; Ref. 18). The AUC from time zero to the time of the final quantifiable sample, AUC\text{total}, was calculated using the linear trapezoidal method. The average C\text{ss} was determined by calculating the average L-778,123 concentration after steady state was attained. CI was estimated as the infusion rate divided by C\text{ss}. The terminal rate constant, k, was determined by log-linear regression analysis of the terminal phase of the plasma concentration-time curve. The t\text{1/2} was calculated as 0.693/k.

Summary statistics were calculated using the JMP version 3.1.6.2 statistical software program (SAS Institute, Cary, NC).

Kinetic linearity was explored by performing a one-way ANOVA of CI versus dose using the Scheffe method for multiple comparisons (19).

**Prenylation of HDJ2** To determine whether FPTase inhibition could be detected and followed in patients receiving L-778,123, the extent of farnesylation of an FPTase substrate, HDJ2 (DNA J homologue) was measured in PBMCs (20). The choice of HDJ2 as a biological marker was based on the following: (a) preclinical experiments demonstrated that HDJ2 prenylation provided a quantitative and consistent indicator of FPTase inhibition; (b) HDJ2 provided significant technical advantages over other markers such as H-ras, particularly because HDJ2 is a more abundant protein than H-ras in virtually all cells and tissues examined; and (c) because the FPTase substrates critical for the biological effects for FPTase inhibitors were not known with any certainty, HDJ2 was thought to be as suitable a marker as any other FPTase substrate.

Blood samples were collected into an 8-ml sodium citrate Vacutainer-CPT tube (Becton Dickinson, Franklin Lakes, NJ) before treatment, 72 h after the start of treatment, and immediately after treatment discontinuation on day 8, and on day 16 during at least courses 1 and 2. Blood samples were centrifuged for 30 min at 1500 \times g at 25°C within 10 min after collection. The mononuclear cell layer was transferred to a 15 ml polypropylene conical tube, and PBS was added. The conical tube was centrifuged at 600 \times g for 10 min at 25°C, supernatant was discarded, and the sample was frozen at -70°C.

Cell pellets were resuspended in 100 \mu l of lysis buffer [20 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% NP40 (v/v), 0.5% sodium deoxycholate, 0.1% SDS, 10 \mu g/ml apro- nin, 2 \mu g/ml leupeptin, 2 \mu g/ml aprotinin, 0.5 \mu M phenylmethyl sulfonyl fluoride, 1 mM DTT], incubated at 4°C for 20 min, and then centrifuged at 10,000 \times g for 10 min. Fifty \mu g of lysate were resolved on precast 8% Tris-Glycine SDS-PAGE mini-gels (Novex, Inc., San Diego, CA), and proteins were transferred to Immobilon-P membranes (Millipore, Inc., Bedford, MA). Membranes were blocked in 2% nonfat dry milk, then probed with an HDJ2 monoclonal antibody (NeoMarkers Inc., no. MS-225-P; distributed by Labvision, Inc., Union City, CA), and then an alkaline phosphatase-conjugated goat antimmunoglobulin IgG (Cappel, Inc., West Chester, PA). Blots were developed with the ECF reagent RPN5785 (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), and HDJ2 immunoreactivity was measured using a Storm 840 PhosphorImager ( Molecular Dynamics, Inc., Sunnyvale, CA). Data were analyzed using ImageQuant Densitometry software ( Molecular Dynamics, Inc.). The nonfarnesylated form of HDJ2 was identified on the basis of its anomalous migration in the SDS-PAGE relative to the farnesylated form; the nonfarnesylated form of HDJ2 displays an apparent molecular weight approximately 5 kDa greater than the farnesylated form of HDJ2 (~44 kDa). The percent of unfarnesylated HDJ2 was determined on the basis of the integrated peak areas of the farnesylated and unfarnesylated forms of HDJ2. The mean percentage of unfarnesylated HDJ2 was determined for each sample from four replicate analyses of each sample.

**Pharmacodynamic Analysis.** The relationship between L-778,123 systemic exposure and the percent inhibition of HDJ2 prenylation was explored. C\text{ss} and AUC\text{total} values served as measures of systemic exposure, and the percentage of unprey-
lated protein on day 4 served as a measure of drug effect. Both simple and sigmoidal \( E_{\text{max}} \) models of drug effect were applied to these relationships using nonlinear least-squares regression (WinNonlin, version 1.1; Statistical Consultants, Apex, NC; Ref. 21). Linear regression models were also applied to the data using the JMP version 3.1.6.2 statistical software program (SAS Institute).

**RESULTS**

**General**

Twenty-five patients, whose characteristics are detailed in Table 1, were treated with 54 total courses of L-778,123 spanning six dose levels. The total numbers of patients and courses administered as a function of the L-778,123 dose level, as well as the overall dose escalation scheme, are depicted in Table 2. Three patients received courses that were not fully evaluable, including a female 60 years of age with renal cell carcinoma who developed a pathological fracture on day 3 of course 2 at the 35-mg/m\(^2\)/day dose level, and a male 20 years of age with an adenocarcinoma of unknown primary who, while concurrently being treated with transthecanse fentanyl, developed grade 3 lethargy and confusion on day 1 of course 1 at the 280-mg/m\(^2\)/day dose level. These symptoms resolved soon after discontinuation of L-778,123 and treatment with naloxone. Although the patient’s analgesic was changed to hydromorphone preceding reinitiation of treatment with L-778,123 at the same dose level 1 week later, grade 3 lethargy and confusion recurred, and treatment with L-778,123 was prematurely terminated. The third patient with a course of treatment that was not fully evaluable developed dose-limiting prolongation of the QTc interval on day 1 of course 1 at the 1120-mg/m\(^2\)/day dose level. Upon discontinuation of L-778,123, the QTc interval normalized, and treatment resumed 1 week later at the 560-mg/m\(^2\)/day dose level without QTc prolongation.

Overall, two patients treated with L-778,123 at the 1120-mg/m\(^2\)/day dose level developed DLT, including the aforementioned patient who had dose-limiting QTc prolongation and another patient who experienced grade 4 thrombocytopenia. In addition, profound grade 2–3 fatigue was noted in two of four patients treated at 1120 mg/m\(^2\)/day, suggesting that chronic treatment of patients with 1120 mg/m\(^2\)/day is not feasible. In contrast, none of eight patients treated at the 560-mg/m\(^2\)/day dose level experienced DLT. Thus, the dose level recommended for disease-directed studies was determined to be 560 mg/m\(^2\)/day.

There was no objective evidence of tumor regression in this study.

**Toxicity**

**Cardiac.** One of 25 patients enrolled onto the study developed dose-limiting cardiac toxicity as defined \textit{a priori}. The patient, a male 75 years of age with pancreatic cancer who was treated with L-778,123 at the 1120-mg/m\(^2\)/day dose level, experienced a 30% prolongation of his QTc interval (from 434 ms to 563 ms) 15 h into the infusion on day 1 of course 1. The patient had no risk factors for QTc prolongation, including hypomagnesemia, hypokalemia, use of concurrent medication associated with QTc prolongation, nor a history of predisposing cardiac or central nervous system disorders. The episode was detected on a cardiac monitor and was not associated with symptoms, arrhythmias, nor a change in the duration of the ventricular complex. The QTc interval normalized after discontinuation of L-778,123, and treatment resumed 1 week later at a reduced L-778,123 dose of 560 mg/m\(^2\)/day without any additional QTc interval prolongation. Isolated, asymptomatic, and transient prolongation of the QTc interval up to 480 ms was also observed in five patients across all dose levels. In addition, a male 57 years of age with pancreatic cancer experienced syncope on day 2 of his first course of L-778,123 at the 280-mg/m\(^2\)/day dose level. An evaluation revealed new-onset atrial fibrillation, which resolved after treatment with adenosine, diliazem, and magnesium sulfate. The L-778,123 infusion, which was interrupted for 24 h during the management of this arrhythmia, was restarted on day 4 without recurrence of atrial fibrillation or related events, and the patient subsequently received three additional uncomplicated courses of L-778,123. QTc prolongation was never evident, and the atrial fibrillation was not felt to be caused by the study medication.

**Hematological.** The distributions of National Cancer Institute grades of neutropenia and thrombocytopenia, and hema-
tological dose-limiting events as a function of L-778,123 dose level are displayed in Table 3. Dose-limiting hematological toxicity was observed in only one patient. The patient, a male 58 years of age with a previously untreated metastatic thyroid cancer, developed grade 4 thrombocytopenia accompanied by grade 3 neutropenia during his first course of L-778,123 at the 1120-mg/m²/day dose level. It was determined that he had progressive disease, and he received no further treatment with L-778,123. Overall, hematological toxicity was modest, with grade 2–3 neutropenia and/or thrombocytopenia noted in five other courses. Interestingly, the only patient to experience myelosuppression (grade 3 neutropenia and thrombocytopenia) of clinical relevance at the 560-mg/m²/day dose level previously had received one day of treatment at the 1120-mg/m²/day dose level, which was discontinued because of the development of QTc prolongation, as discussed above.

Miscellaneous. Among the other toxicities observed subsequent to treatment with L-778,123, grade 1–3 nausea and/or vomiting were the most common, occurring during nine courses. Most events (seven courses) were mild to moderate (grade 1–2) in severity, well managed, and subsequently prevented with serotonin receptor antagonists, and therefore no event was considered dose-limiting.

Fatigue was noted in eight courses. All episodes were classified as grade 1–2, except for two grade 3 events in two patients treated at the 1120-mg/m²/day dose level. Although there were other potentially confounding etiological factors, including disease progression, that may have been partly responsible for the fatigue, the severity and consistent nature of this effect suggested that the events were drug-related and contributed to the perception that the 1120-mg/m²/day dose level was not suitable for chronic administration according to the criteria defined a priori. Somnolence and confusion of grades 2 and 3 severity occurred in one patient each at the 280- and 560-mg/m²/day dose levels, respectively. Both patients had been receiving concurrent treatment with opioid analgesic medications. The patient at the 280-mg/m²/day dose level had a rapidly changing narcotic requirement and developed progressive lethargy and confusion on day 2. This event was felt to be caused by either the narcotics alone, or the combination of narcotics and L-778,123. The patient at the 560 mg/m²/day dose level was a noncompliant diabetic with hyperglycemia, who was subsequently found to have brain metastases. Neither of these events were classified as dose limiting.

Although all 25 patients had extensive serial visual examinations, no changes in funduscopic exam or visual acuity were found. Furthermore, asymptomatic, fully reversible decrements in parameters indicative of rod and cone function were evident on electroretinography in only four patients across all dose levels [140 mg/m²/day (one patient), 280 mg/m²/day (two patients), and 1120 mg/m²/day (one patient)]. The first patient noted to have electroretinogram changes was a woman 76 years of age with colorectal cancer who was treated at the 140 mg/m²/day dose level. Although the asymptomatic B-wave changes observed on day 8 had resolved by day 22, course 2 was administered at a reduced dose of 70 mg/m²/day, and no additional electroretinogram changes were observed. The other patient in whom scheduled L-778,123 treatment was affected by the electroretinogram was a male 59 years of age with colorectal carcinoma who developed 29–36% decrements in A and B wave amplitudes on day 20 of his first course of L-778,123 at the 280-mg/m²/day dose level. After a 1-week treatment delay between courses 1 and 2, these abnormalities resolved, and they did not recur during a second course administered at the same dose.

Pharmacological Studies

Blood sampling was performed in 23 (92%) of 25 patients to evaluate the pharmacological behavior of L-778,123. Pharmacokinetic parameters could not be determined for one patient at each of the 280- and 1120-mg/m²/day dose levels, because treatment was discontinued prematurely. In addition, pharmacokinetic parameters were not reflective of a 7-day infusion for one patient at the 560-mg/m²/day dose level, because the infusion was interrupted for 24 h after the patient developed atrial fibrillation on day 2. For another patient at the 560-mg/m²/day dose level, insufficient sampling on day 8 precluded a reliable determination of the terminal half-life. Pertinent pharmacokinetic parameters as a function of dose are listed in Table 4, and a representative concentration-time curve is shown in Fig. 2. At the 560-mg/m²/day dose level, CSs averaged 8.09 ± 3.11 μM, which exceeded the IC50 values (range, 0.07–5.35 μM) for the spectrum of human cancer cell lines evaluated in preclinical studies (16). Within the range of doses studied, there were no significant differences (P = 0.133) among the Cl values for each dose level, suggesting that L-778,123 displays linear drug elimination kinetics (19). Across all dose levels, Cl averaged 106.4 ± 45.6 ml/min/m², and the mean harmonic t1/2 was 2.8 ± 1.0 h. On the basis of limited data from day 10 and beyond, there

### Table 3 Hematologic toxicity

<table>
<thead>
<tr>
<th>L-778,123 dose level (mg/m²/day)</th>
<th>Total no. of Patients</th>
<th>Courses</th>
<th>Neutropenia Grade</th>
<th>Thrombocytopenia Grade</th>
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may have been a phase with a longer half-life, but this could not be adequately characterized. By day 14, L-778,123 plasma concentrations were generally below the limit of quantification for the assay.

Stratification of pharmacokinetic parameters according to DLT was limited by the small number of patients treated at the 1120-mg/m²/day dose level. TheCss (19.5 μM) value determined for the patient who experienced dose-limiting thrombocytopenia approximated the Css (range, 16.4–18.4 μM) values determined for the two patients who did not experience DLT. Steady-state parameters could not be determined for the patient who experienced dose-limiting QTc prolongation, because the infusion was discontinued before adequate sampling could be obtained. However, 9 h after QTc prolongation necessitated discontinuation of the infusion, a plasma concentration of 71.1 μM was reached. This value was >300% of the Css values for the other three patients treated at the 1120-mg/m²/day dose level.

### HDJ2 Prenylation Studies

The effects of L-778,123 treatment on HDJ2 prenylation in PBMCs were determined during 40 courses in 21 patients. The analysis could not be completed in one patient each treated at the 280- and 1120-mg/m²/day dose levels, because treatment was discontinued prematurely due to toxicity. In two additional patients treated with L-778,123, 1120 mg/m²/day, protein yields were insufficient for reliable assay quantification. The average percentages of HDJ2 protein in its unprenylated form as a function of both dose level and treatment day in 21 patients are shown in Fig. 3. L-778,123 treatment resulted in an increase in the percentage of unprenylated HDJ2 on treatment day 4 in all patients. Overall, the percentage of unprenylated protein peaked on day 4, with negligible fluctuation observed from this time until the next assessment performed immediately after treatment discontinuation on day 8. The magnitude of the inhibitory effects of L-778,123 on HDJ2 prenylation seemed to increase as the dose of L-778,123 was increased, reaching a plateau at 560 mg/m²/day; however, the small number of patients treated at each dose level limited an adequate analysis of dose effect. At the recommended Phase II dose, 560 mg/m²/day, the mean percentage of HDJ2 protein in the unprenylated form increased from 1.41% ± 1.71% before treatment to 28.76% ± 6.10% midtreatment (day 4) and 30.86% ± 4.96% immediately after treatment (day 8). The effect of L-778,123 on HDJ2 prenylation was short lived, with the percentage of unprenylated protein generally returning to pretreatment levels 1 week after treatment (day 16). At the 560-mg/m²/day dose level, the percentage of unprenylated HDJ2 declined to 2.28% ± 2.11% one week after drug discontinuation (day 16). The inhibitory effects of L-778,123 on HDJ2 prenylation seemed to be related to L-778,123 Css, although the relationship was not adequately described by either simple or sigmoidal Emax models ($r^2$ ≤ 0.29 and $r^2$ ≤ 0.34, respectively) or by linear models ($r^2$ ≤ 0.38). The extent of HDJ2 prenylation could not be related to toxic events, because there were insufficient toxic events for valid pharmacodynamic analyses.

### DISCUSSION

This Phase I study was designed to evaluate the feasibility of administering L-778,123 as a continuous infusion over 24 h for 7 days every 3 weeks. At the highest dose evaluated in the present study, 1120 mg/m²/day, intolerable myelosuppression and QTc prolongation were noted, and two of four patients developed profound fatigue. In contrast, no DLT was evident in eight patients treated at the next lower dose level, 560 mg/m²/
pretreatment, during L-778,123 treatment, immediately after discontinuation of treatment (day 8), and after discontinuation of the infusion (day 16) as a function of L-778,123 dose level.

Fig. 3 Mean (±SD) percentage of unprenylated HDJ2 in PBMCs pretreatment (day 0), during L-778,123 treatment (day 4), immediately after discontinuation of treatment (day 8), and after discontinuation of the infusion (day 16) as a function of L-778,123 dose level.

day, which was determined to be the recommended dose for subsequent Phase II evaluations. More protracted (2–4 week) continuous i.v. administration schedules of L-778,123 were evaluated in concurrent studies performed at other institutions (22). Similar DLTs of myelosuppression and QTc prolongation were observed at 840 mg/m²/day for 14 days, and, as a result, it was decided not to explore intermediate dose levels using the 7-day continuous infusion schedule. The recommended dose of L-778,123 is 560 mg/m²/day irrespective of whether the agent is administered as a protracted infusion continuously for 1, 2, or 4 weeks, suggesting that these toxicities may be related to dose and/or peak concentration (22).

The principal adverse effects and dose-limiting toxicities of L-778,123, myelosuppression and prolongation of the QTc interval, were similar to those observed in preclinical toxicology studies of L-778,123. In the current Phase I trial, dose-limiting thrombocytopenia occurred in one course at the 1120-mg/m²/day dose level, whereas hematological toxicity was infrequent and only mild to moderate in severity at the recommended dose of 560 mg/m²/day. Similar observations have been made in evaluations of more prolonged L-778,123 administration schedules, in which the 840-mg/m²/day-for-14-doses dose level has been associated with grade 4 neutropenia (22). Interestingly, myelosuppression, particularly neutropenia and thrombocytopenia, have also been the principal DLTs of several oral nonpeptidomimetic inhibitors of FPTase, including the tricyclic FPTase inhibitor SCH66336 and the quinolone R115,777 administered on protracted once- or twice-daily dosing schedules, indicating that myelosuppression may be a “class effect,” and that hematopoiesis may be dependent on protein prenylation (23, 24).

Although the precise mechanism responsible for the effect of L-778,123 on the QTc interval is not known, it is most likely attributable to direct drug effects on the IKr cardiac current channel (25), rather than caused by indirect effects through the inhibition of farnesyltransferase. Limited toxicity data suggest that the cardiac effects of L-778,123 may be related to drug exposure, because the plasma concentration in the solitary patient who developed dose-limiting QTc prolongation at the 1120-mg/m²/day dose level was 71 µM shortly after drug discontinuation on day 2, which was much greater than the Cₘₐ values in the other three patients at that dose level (range, 16.4–19.5 µM). Furthermore, at the recommended dose level of 560 mg/m²/day, the mean Cₘₐ was 8.09 ± 3.11 µM, and no significant QTc prolongation was observed.

Although not defined as dose-limiting, severe fatigue, somnolence, and confusion were observed in some patients treated with L-778,123. Severe (grade 3) fatigue was experienced by two of four patients treated with L-778,123, 1120 mg/m²/day, whereas grade 1–2 fatigue was commonly experienced by patients treated at the lower dose levels. This degree of fatigue is similar to that described in early studies of the nonpeptidomimetic FPTase inhibitors SCH66336 and R115,777 (26, 27) and, although highly speculative, may be attributed in part to drug-induced inhibition of farnesyl transferase of phospholipase kinase α and β, skeletal muscle proteins that function in glycogen metabolism (3). However, there were no specific manifestations of muscle weakness, inflammation, or loss in patients treated in early studies of FPTase inhibitors. More exceptional than the fatigue were the grade 2–3 somnolence and confusion experienced by one patient each at the 280- and 1120-mg/m²/day dose levels. The somnolence and confusion were initially thought to be caused by interactions between narcotic analgesics and L-778,123, although no pharmacokinetic interaction between L-778,123 and opioids was established. In the affected patients, the plasma opioid concentrations were consistent with the doses of opioids administered, and L-778,123 Cₘₐ values were similar to those documented in other patients treated at the respective dose levels.

All patients were monitored extensively for retinal toxicity because farnesylated proteins localized within the rod and cone photoreceptors play an essential role in phototransduction, the process by which light is converted to an electrical signal (28). In the present study, routine eye examinations failed to reveal any abnormalities, whereas serial electroretinography demonstrated transient asymptomatic effects. Routine eye examinations and retinal photography performed in early clinical studies of other far- nesyl transferase inhibitors have not revealed ocular toxicity (23, 24, 26, 27), however, electroretinography may be required to detect alterations in phototransduction. Nonetheless, the small and reversible changes observed with electromyography in this study were not associated with any clinical sequela.

Pharmacokinetic studies revealed that the plasma L-778,123 Cₘₐ increased proportionally with L-778,123 dose. At the recommended dose of 560 mg/m²/day, the mean Cₘₐ was 8.09 ± 3.11 µM, a value that not only exceeded the Kᵢ of FPTase for competition with Ras substrate, but also exceeded the IC₅₀ values for the spectrum of human cancer cell lines evaluated in preclinical studies (16). Patients were effectively exposed to the Cₘₐ for most of the 7-day infusion because steady-state was
attained ~3 h into the infusion, and plasma concentrations declined rapidly after treatment. Protracted administration schedules have been explored in an effort to increase L-778,123 exposure (22).

To detect and quantify potentially relevant biological effects of L-778,123 as well as to explore the possibility of developing a pharmacodynamic assay to serially assess functional drug effects, prenylation of the chaperone protein HDJ2 was measured in PBMCs. Indeed, the inhibition of HDJ2 prenylation was consistently detected midtreatment (day 4), and the magnitude of this effect seemed to be dose-related, reaching a plateau at L-778,123 doses ≥560 mg/m²/day. The maximal effect of L-778,123 on FPTase inhibition could not be precisely determined because unacceptable toxicity at the 1120 mg/m²/day dose level precluded additional dose escalation. More importantly, the inhibitory effects of L-778,123 on HDJ2 prenylation were short-lived, with full recovery noted within 1 week after discontinuation of treatment. The preliminary results of these studies suggest that additional dose escalation or more protracted infusions would likely fail to enhance the magnitude of the inhibitory effects of L-778,123 on protein prenylation, although more protracted infusions would likely increase the duration of the inhibitory effects of L-778,123 on protein prenylation. An important caveat is that the prenylation status of the HDJ2 chaperone protein, particularly in PBMCs, may not reflect the prenylation status of Ras and other “true” target proteins in tumor tissues. Nevertheless, the HDJ2 prenylation assay performed in this study seems to be a logical and internally consistent pharmacodynamic marker to evaluate the inhibitory effects of FPTase-targeted therapeutics in the course of therapeutic development.

Although L-778,123 was rationally developed to inhibit mutationally activated Ras, the mechanism of action of FPTase inhibitors is being reevaluated. Given the current ambiguity surrounding the mechanism of action of FPTase inhibitors, this Phase I study was designed to include patients with any advanced solid malignancy, regardless of ras mutation status. No objective tumor responses were observed. The lack of antitumor activity observed in this study may be attributable to the high specificity of L-778,123 for FPTase over GGPTase, a property observed in this study may be attributable to the high specificity of L-778,123 for FPTase over GGPTase, a property that was originally thought to limit toxicity. However, in the setting of FPTase inhibition, GGPTase may be able to restore the function of proteins that are normally farnesylated (1–3, 12–14, 29). It is conceivable that less specific compounds capable of inhibiting both FPTase and GGPTase may be more effective.

The results of the present study indicate that L-778,123 administered at a dose of 560 mg/m²/day as a continuous 7-day infusion every 3 weeks is well tolerated, and that biologically relevant plasma drug concentrations are achieved and sustained for the duration of the infusion. Although additional development of L-778,123 is not planned, this study demonstrates the implementation of the HDJ2 prenylation assay as a marker of the biological effects of an FPTase inhibitor.

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A Phase I and Pharmacological Study of the Farnesyl Protein Transferase Inhibitor L-778,123 in Patients with Solid Malignancies

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