A Phase I Trial of the Dual Farnesyltransferase and Geranylgeranyltransferase Inhibitor L-778,123 and Radiotherapy for Locally Advanced Pancreatic Cancer

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ABSTRACT

Purpose: **Preclinical and clinical studies have demonstrated that inhibition of prenylation can radiosensitize cell lines with activation of Ras and produce clinical response in patients with cancer. The aim of this study was to determine the maximally tolerated dose of the dual farnesyltransferase and geranylgeranyltransferase I inhibitor L-778,123 in combination with radiotherapy for patients with locally advanced pancreatic cancer.**

Experimental Design: **L-778,123 was given by continuous intravenous infusion with concomitant radiotherapy to 59.4 Gy in standard fractions. Two L-778,123 dose levels were tested: 280 mg/m^2/day over weeks 1, 2, 4, and 5 for dose level 1; and 560 mg/m^2/day over weeks 1, 2, 4, 5, and 7 for dose level 2.**

Results: **There were no dose-limiting toxicities observed in the eight patients treated on dose level 1. Two of the four patients on dose level 2 experienced dose-limiting toxicities consisting of grade 3 diarrhea in one case and grade 3 gastrointestinal hemorrhage associated with grade 3 thrombocytopenia and neutropenia in the other case. Other common toxicities were mild neutropenia, dehydration, hyperglycemia, and nausea/vomiting. One patient on dose level 1 showed a partial response of 6 months in duration. Both reversible inhibition of HDJ2 farnesylation and radiosensitization of a study patient-derived cell line were demonstrated in the presence of L-778,123. K-*RAS* mutations were found in three of the four patients evaluated.**

Conclusions: **The combination of L-778,123 and radiotherapy at dose level 1 showed acceptable toxicity in patients with locally advanced pancreatic cancer. Radiosensitization of a patient-derived pancreatic cancer cell line was observed.**

INTRODUCTION

Pancreatic cancer will have an estimated incidence of 31,860 in the United States in 2004 with a nearly equal mortality rate (1). Combination chemoradiation is considered a standard treatment for the 40% of these patients who present with locally advanced, unresectable disease (2, 3). Chemoradiation results in a median survival of 7–11 months, which represents a modest improvement over either therapy alone (3). The reasons for the relatively poor response of pancreatic cancer to treatment are not known but may relate to prevalent genetic changes in these tumors.

A number of molecular modifications found in pancreatic cancer lead to increased signaling through the Ras pathway including increased phosphorylated Akt (P-Akt) and epidermal growth factor receptor (EGFR) expression (4, 5). The *RAS* oncogenes encode a family of small GTP-binding proteins that act at the cytoplasmic side of the cell membrane by transducing signals from cell surface receptor tyrosine kinases to a cascade of downstream effectors. The various pathways lead to cellular responses such as proliferation, differentiation, and apoptosis (reviewed in Ref. 6). Activating point mutations in *RAS* have been identified in nearly 25% of all human tumors, with the majority of mutations found in the K-*RAS* isotype (7). Pancreatic adenocarcinoma, in particular, has a 75–90% frequency of K-*RAS* mutations found predominantly at codon 12 (8–10). Ras was shown to play a role in radioresistance in NIH3T3 cells over 15 years ago (11). Since that time, studies using both animal and

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human tumor cells have demonstrated that activation of H-, N-, and K-Ras leads to increased radioresistance (12–15).

To properly associate with the cell membrane, the Ras proteins must first undergo prenylation (16, 17). This addition of a 15-carbon farnesyl isoprenoid to a cysteine near the COOH terminus of the protein is carried out by the enzyme farnesyltransferase (FTase). For certain Ras isotypes, a 20-carbon geranylgeranyl group is added to this cysteine by geranylgeranyltransferase I when farnesylation is inhibited (18).

The FTase inhibitors (FTIs) are compounds developed to inhibit FTase and have been the focus of numerous preclinical and clinical studies (reviewed in Ref. 19). *In vitro* and *in vivo* studies of animal and human tumor cells have established that FTIs can inhibit growth in a variety of cell types (20–22). In addition, FTIs have been shown to reverse cellular radiation resistance in rat embryo fibroblasts and human xenograft tumors that express *RAS* activated by mutation (23–25). Whereas inhibition of mutated *RAS* activation was the original target for these therapeutics, evidence now suggests that the effects of FTIs on other molecular targets play a significant role in FTI radiosensitization and cell growth inhibition (26–28).

L-778,123 inhibits both FTase and geranylgeranyltransferase I (29). The drug has been used in two human trials as single-agent therapy for solid tumors showing dose-limiting toxicities (DLTs) of (*a*) corrected QT interval (QT_c) prolongation and grade 4 neutropenia at 840 mg/m^2/day in a 2-week continuous infusion trial and (*b*) grade 4 thrombocytopenia, significant QT_c prolongation, and profound fatigue at 1120 mg/m^2/day in a trial investigating 7-day continuous infusion every 3 weeks (30, 31). Our group has previously studied the combination of L-778,123 and radiation in a Phase I study of non-small cell lung cancer (NSCLC) and head and neck cancer (HNC) patients (32). Among the nine patients enrolled, five (three NSCLC and two HNC patients) had a complete response, and one patient with NSCLC had a partial response. In the two dose levels investigated (280 and 560 mg/m^2/day), a single patient, on the higher dose level, experienced a DLT of grade 4 neutropenia. With continued interest in the use of prenylation inhibitors as radiosensitizers, we present here the data from a separate cohort of patients in the Phase I study with locally advanced pancreatic cancer for whom dose escalation occurred independently of the NSCLC/HNC patients. The primary objective of this study was to establish the DLT and maximally tolerated dose (MTD) of the combination of L-778,123 and radiation in patients with locally advanced pancreatic cancer.

PATIENTS AND METHODS

Patient Selection. Patients with histologically documented pancreatic cancer that, in the opinion of the investigator, required radiation therapy were enrolled in this study at four institutions. Additional inclusion criteria were as follows: (*a*) age ≥18 years; (*b*) measurable disease; (*c*) Eastern Cooperative Oncology Group performance status of 0 or 1; (*d*) life expectancy of >3 months; and (*e*) signed consent. Patients were excluded if they (*a*) had received oral steroids, immunological therapy, radiation therapy, tumor resection, or chemotherapy or had participated in another investigational drug study within 4 weeks of the study; (*b*) were predicted to require endocrinologic, immunological, chemotherapeutic, or surgical therapy during the study period; (*c*) had received radiation to >25% of their bone marrow or received stem cell rescue after chemotherapy; (*d*) had abnormal organ function including coagulation (international normalized ratio or activated partial thromboplastin time > 1.2× normal) or abnormal hematological (absolute neutrophil count < 1,500/mm^3, platelet count < 100,000/mm^3, or hemoglobin < 9 g/dl), hepatic (bilirubin > 1.5× normal, alanine/aspartate aminotransferase > 2× normal), and renal/metabolic values (creatinine > 1.5× normal or serum sodium, potassium, calcium, or magnesium > 10% outside the normal range); (*e*) had a history of significant ventricular dysrhythmias or QT_c abnormalities requiring medication including $QT_c \geq 440$ ms; (*f*) had significant cardiac history including a myocardial infarction within 1 year of study or recent unstable angina or heart failure; (*g*) required potent inducers of CYP3A; (*h*) required β-hydroxy-β-methylglutaryl CoA reductase inhibitors, with the exception of pravastatin or fluvastatin; (*i*) had significant retinal disorder; (*j*) had an active central nervous system malignancy or seizure disorder; (*k*) had a cumulative doxorubicin dose of ≥450 mg/m^2; (*l*) had an active infection; (*m*) were pregnant or lactating; (*n*) were known HIV positive; (*o*) had a significant psychiatric disorder or recent history of drug or alcohol abuse; or (*p*) had a significant drug or latex allergy.

Study Design. This was a Phase I, nonrandomized, dose-escalation study of the FTI L-778,123 plus radiation therapy in patients with locally advanced pancreatic cancer. The primary end points were to evaluate toxicity and determine the MTD of L-778,123 when used concurrently with radiotherapy. The study also enrolled patients with NSCLC and HNC in a separate dose-escalation cohort reported previously (32). Informed consent according to institutional and federal guidelines was obtained from every enrolled patient before the onset of therapy.

Two dose levels of L-778,123 were studied. Dose escalation was not permitted for individual patients. Selection of the two dose levels was based on clinical and preclinical data. Dose level 1 (280 mg/m^2/day) was expected to achieve sufficient plasma concentrations of L-778,123 to inhibit farnesylation and radiosensitize cells, and dose level 2 (560 mg/m^2/day) had been shown to be well tolerated in a dose-escalation trial (31). The goal was to provide radiosensitization throughout the 7-week course of radiotherapy. At the outset of the trial, clinical data had shown the tolerability of a schedule of two 2-week continuous infusions with a 1-week break between the two. This led to an initial design of giving the drug by continuous infusion over 7 days for weeks 1, 2, 4, and 5. Data from a parallel study, made available after completion of the first cohort in this study, showed that L-778,123 as a single agent could be given over a more prolonged course, prompting a change in design for the dose level 2 cohort to receive drug over weeks 1, 2, 4, 5, and 7. At least three patients were to be enrolled at each level. Enrollment at dose level 2 was initiated only if no DLTs were observed in the dose level 1 cohort for a minimum of 6 weeks after completion of radiation. If one of the three patients experienced a DLT, three additional patients were to be enrolled at that dose level. Patients continued to be enrolled on the first dose level until the required cohort had been observed for 6 weeks after therapy.

DLTs were generally defined by the National Cancer In-

stitute's Common Toxicity Criteria as grade 3 toxicities of any duration. The following specific criteria were exceptions to this rule and were DLTs: (*a*) irreversible grade 2 neurotoxicity; (*b*) grade 4 pancreatitis; (*c*) grade 4 radiation dermatitis; (*d*) certain hematological toxicities (any grade 4 toxicity, grade 3 toxicity of >1 week in duration, or irreversible grade 2 toxicity); (*e*) QT_c prolongation ≥ 490 ms with at least a 10% increase from the baseline or an increase of ≥80 ms from baseline regardless of the absolute value; and (*f*) interruption in radiation therapy for more than 2 weeks or two interruptions, each of which lasted at least 1 week.

L-778,123 Administration. L-778,123 was provided by Merck Research Laboratory (Rahway, NJ) as a 10 mg/ml solution in saline. Three-day supplies were prepared in 250 ml of normal saline for patient administration. Each patient had a central venous catheter placed before therapy. Beginning on day 1 of the radiotherapy, the drug was administered as a continuous infusion at 3.4 ml/h with a Deltec CADD-PLUS model 5400 infusion pump (SIMS Deltec, Inc., St Paul, MN).

External Beam Radiation. Radiation was started on day 1 after initiation of the L-778,123 infusion. The dose was prescribed at mid-separation on the central ray for two opposed, equally weighted beams. For all other arrangements, the dose was prescribed at the center of the target area or at the intersection of the central rays of the beams. Computed tomography-based simulation was required for all fields, and the target volumes were defined by shaped ports with custom-made blocks or multi-leaf collimators. All fields required portal verification, and the patient had to be reproducibly immobilized. Source-axis distance techniques were used, and each field was treated at every session. Megavoltage equipment was required with minimum peak photon energies of 6 MV.

The total radiation dose was 59.4 Gy administered in 1.8 Gy daily fractions five times per week. The gross tumor and areas at risk for microscopic metastasis were treated to 45 Gy followed by an additional 14.4 Gy in the same fractionation to gross tumor with a margin of 1.5–2.0 cm. Every attempt was made to limit the maximal spinal cord dose at any level to ≤40 Gy. Attempts were also made to exclude one kidney from the field, but if both total kidneys were in the field, the dose for one kidney was limited to 15 Gy. Additionally, no more than 50% of the hepatic volume was to exceed 30 Gy.

Patient Evaluation. On day 1, vital signs and a 12-lead electrocardiogram for evaluation of the QT_c interval were obtained before, 0.5 h, and 3 h after initiation of the L-778,123 infusion. A cardiology consultation and inpatient monitoring were required for a QT_c of ≥440 ms but <490 ms. If the QT_c was consistently ≥490 ms, L-778,123 was discontinued.

Patients were seen on a weekly basis during radiotherapy and then reevaluated 3 and 6 weeks after the completion of therapy. Radiological assessments of tumor response were first obtained within 12 weeks of completion of the radiotherapy and were obtained regularly thereafter. The responses were based on the best radiological response achieved, observed at any follow-up scan. Responses were classified into four categories based on two scans separated by at least 4 weeks. Complete response was defined as disappearance of all measurable disease over the two-scan period. Partial responses were defined as a ≥50% reduction in the product of the perpendicular diameters of all measurable lesions, as well as the absence of any new lesions over the given period. Progressive disease was defined as an increase of ≥25% in the product of the perpendicular diameters of all measurable lesions or the appearance of new disease during the same period. Finally, stable disease was defined as disease failing to fulfill criteria for partial response or progressive disease.

Farnesylation Assay. The extent of farnesylation inhibited by L-778,123 was measured in peripheral blood mononuclear cells (PBMCs) using the FTase substrate HDJ2. Because the specific role of farnesylation in radioresistance is unknown, HDJ2, an abundant DnaJ homolog, serves as a reproducible and quantitative biological marker for global inhibition of FTase (31, 33). Samples were collected within 72 h before initiation of L-778,123 and then collected on days 8, 15, 22, 29, and 36 for patients on dose level 1 and on days 8, 15, 22, 29, 36, 43, and 50 for patients on dose level 2. A final sample was collected approximately 3 weeks after completion of the radiotherapy in both groups.

A detailed description of the methods used is published elsewhere (31). Briefly, the blood samples were collected in sodium citrate, and the mononuclear cells were removed. These cells were lysed, and the lysate was resolved on SDS-PAGE before transfer to membranes for immunoblotting with HDJ2 monoclonal antibody. Farnesylated and nonfarnesylated HDJ2 were measured quantitatively using the integrated peak areas from the fluorescence signal. The nonfarnesylated form of HDJ2 was identifiable based on an apparent molecular weight that was 5000 greater than that of the farnesylated form on SDS-PAGE. The percentage of nonfarnesylated HDJ2 is calculated relative to the sum of the farnesylated and nonfarnesylated peaks. Replicate analyses ($n = 4$) were performed for each sample to minimize measurement error. Interpatient mean percentage of nonfarnesylation was calculated for each dose level and plotted.

Primary Cell Culture. After receiving consent from the patient and appropriate institutional approval, a sample of malignant ascites from a patient on therapy was collected to establish a primary cell line (the PancM cell line). After centrifugation of the fluid, the cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (HyClone, Logan, UT), penicillin (100 units/ml; Life Technologies, Inc., Rockville, MD), streptomycin (100 mg/ml; Life Technologies, Inc.), 4.5 g/liter dextrose (Fisher Scientific, Pittsburgh, PA), and 1 mM sodium pyruvate (Life Technologies, Inc.). All cultures were maintained at 37°C in water saturated with 5% CO_2 and 95% air.

Radiation Cell Survival Studies. PancM cells growing in log phase were used in all clonogenic survival experiments. The cells were pretreated with either 5 μM L-778,123 or an equal volume of the drug diluent DMSO. The pretreated and control cells were harvested after 24 h, and single cell suspensions were plated in L-778,123 or carrier, respectively. Using a Mark 1 cesium irradiator (J. L. Shepherd, San Fernando, CA) at a dose rate of 1.6 Gy/min, the cells were irradiated with 1–6 Gy at ambient room temperature. The growth medium for both drug-treated and control cells was replaced with drug-free medium 24 h after irradiation. The plates were stained for colony formation at 14–21 days, and the surviving fraction was determined based on the plating efficiency for unirradiated cells from

each treatment type. For each radiation dose, the surviving fraction was defined as the number of colonies formed divided by the number of cells plated multiplied by the plating efficiency of unirradiated controls.

Molecular Markers. With institutional approval and patient consent, samples of pancreatic tumor from the diagnostic biopsy were collected from four study patients. Analysis of the samples was performed for *RAS* mutational status, P-Akt expression, and EGFR expression.

The *RAS* status was determined by PCR amplification specific for H-, K-, and N-*RAS* mutations at codons 12, 13, and 61, with confirmation by direct sequencing of the amplified portion.

The expression of P-Akt and EGFR was determined by immunohistochemical staining as described previously (34). Briefly, for EGFR, tissue slides were deparaffinized, rehydrated, and antigen unmasked with pronase treatment before incubation with primary EGFR antibody (DAKO, Carpinteria, CA). After immunostaining, slides were counterstained with hematoxylin and mounted for examination. The EGFR staining was classified as negative, 1+, 2+, or 3+, based on the intensity and pattern of the membrane staining.

For P-Akt evaluation, following deparaffinization and antigen unmasking, the tissue was incubated with the Ser^{473} antibody (New England Biolabs, Beverly, MA). Detection was accomplished with the secondary antirabbit biotinylated antibody (Vector, Burlingame, CA) followed by enzyme conjugate StreptABC complex horseradish peroxidase reagent (DAKO) and chromogen (DAKO) incubation. Slides were mounted and scored as described above.

RESULTS

Patient Characteristics. Twelve patients (Table 1) were enrolled in the study (four women and eight men). The median age was 59 years, with a range of 39–73 years. Eight patients were enrolled at dose level 1, and four patients were enrolled at dose level 2. Whereas no patient had received prior chemotherapy, radiation therapy, or surgical resection, six had undergone palliative biliary diversion procedures.

Toxicities. The number of patients experiencing toxicities for each dose level is shown in Table 2. The patient's most severe toxicity thought to be at least possibly related to therapy is reported for each category. Two patients on the second dose level (560 mg/m^2/day) experienced DLTs. One had grade 4 neutropenia and grade 3 thrombocytopenia, associated with a grade 3 upper gastrointestinal (GI) hemorrhage from a gastric ulcer believed to be outside the radiation field. In addition, this patient had minor (≤grade 2) episodes of proteinuria, hematuria, partial thromboplastin time elevation, electrolyte abnormalities, and hyperglycemia. The L-778,123 dose in this patient was lowered to level 1 after the hemorrhagic episode, and he completed therapy without further incident. The patient died from progressive disease approximately 6 months after entering the study.

The other patient with a DLT experienced two episodes of grade 3 diarrhea: one brief episode while on therapy, which resolved with standard treatment; and a second, more prolonged course in the posttreatment period that showed modest improvement with medication. In addition to the diarrhea, she experienced mild neutropenia, aminotransferase elevations, alkaline phosphatase elevation, proteinuria, hematuria, thrombocytopenia, protime prolongation, nausea, vomiting, hyperglycemia, and dehydration. She completed therapy and had a GI evaluation suggesting a multifactorial etiology for the diarrhea including malabsorption.

Several other patients on dose level 1 had >grade 2 toxicities felt to be unrelated to the therapy. One patient had a grade 3 small bowel obstruction, which was thought to be secondary to an adhesion from a prior operation, requiring hospitalization for bowel rest and total parenteral nutrition. The patient proceeded to develop increasing ascites requiring multiple paracenteses that showed recurrent disease. He was taken off study for progressive disease after receiving 39.6 Gy of radiation. In addition, this patient had grade 3 hypoalbuminemia associated with the small bowel obstruction as well as mild hematuria, hyperglycemia, electrolyte abnormalities, and protime prolongation. The patient died 2 months after study entry.

A second patient on dose level 1 developed grade 3 hyponatremia as a result of volume depletion while on therapy. She was subsequently admitted for hydration and on work up

Table 1 Patient characteristics

	No.
Gender	
Male	8
Female	4
Age (yrs)	
Mean	59
Range	39–73
Dose (mg/m^2/day)	
280	8
560	4
Prior treatment	
Palliative intervention	6
Chemotherapy	0

Table 2 Number of patients with toxicity events by dose level

	280 mg/m^2/day (n = 8)				560 mg/m^2/day (n = 4)			
	Grade				Grade			
Toxicity*	1	2	3	4	1	2	3	4
Hematological	2	1	1			1		1†
Gastrointestinal		6	1			2	1†	
Electrolytes	3		2		2			
Other		2				1		
Proteinuria/hematuria	3				2			
Cardiac	2				1			
Hepatic	1	1	1		1			
Coagulation	2		1		1		2†	
Constitutional	1	2						
Infectious			1			1		

NOTE. Each patient counted once per toxicity category, with the severest toxicity being recorded.

* Toxicity was defined according to the National Cancer Institute Common Toxicity Criteria.

† A DLT.

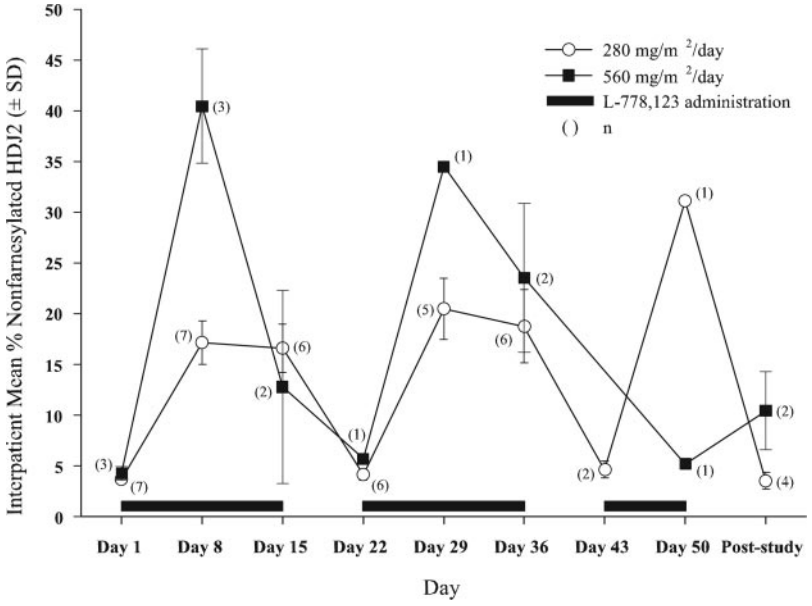

Fig. 1 Inhibition of FTase action on HDJ2 with L-778,123. PBMC-derived farnesylated HDJ2 analysis at one week intervals for 280 mg/m^2/day (○) and 560 mg/m^2/day (■) L-778,123 treatments. Each point represents the interpatient mean based on (n) patients with error bars representing the SD. Treatment with L-778,123 showed a reversible inhibition of HDJ2 farnesylation (an increase in nonfarnesylated HDJ2) with both dose levels.

was found to have grade 3 neutropenia with mild thrombocytopenia and anemia felt to be the result of the therapy. The neutropenia resolved, but her performance status continued to decline, and computed tomography confirmed disease progression. The volume depletion and hyponatremia associated with this event were felt to be a result of disease progression and not of therapy. She was removed from the protocol after receiving 27 Gy of radiation and died 5 days later, 1 month after entering the study. Mild diarrhea and hyperglycemia were also observed.

A third patient on therapy at dose level 1 experienced an episode of grade 3 hyperglycemia that was not felt to be related to the study therapy. She also experienced grade 2 scaling and hyperpigmentation of the hands, nausea and vomiting, anemia, electrolyte abnormalities, fatigue, edema, and dizziness.

A patient treated at dose level 1 had to be withdrawn from therapy for grade 3 thrombosis and grade 3 non-neutropenic bacterial infection at the site of the central line 2 weeks after starting therapy. These toxicities were not felt to be related to therapy. The patient had also experienced mild nausea, vomiting, and multiple episodes of grade 1 sinus tachycardia and bradycardia. He died of disease progression 5 months after entering the study.

Finally, a patient on dose level 2 had to be withdrawn from the study after 4 weeks of therapy due to grade 3 GI hemorrhage from preexisting hemorrhagic gastritis. Early in the therapy, the patient experienced grade 2 nausea and vomiting requiring a short break in treatment but was otherwise without toxicities. Four months after enrolling in the study, the patient died of progressive disease.

Otherwise, toxicities were mild, consisting most frequently of nausea and vomiting, dehydration, neutropenia, hyperglycemia, electrolyte abnormalities, hematuria, and proteinuria. One patient on dose level 1 experienced mild QT_c prolongation, a toxicity thought to be unique among FTIs to L-778,123.

Response. Eight patients completed therapy and had evaluable disease by radiological measurements within 12 weeks of completion of radiotherapy. Of the four patients who discontinued the study, two discontinued for disease progression as noted above, and two discontinued for toxicities consisting of hemorrhagic gastritis and bacterial infection, respectively. One patient on dose level 1 had radiologically determined partial response maintained for 6 months from the beginning of therapy. The patient had disease progression at 7 months by radiographic evaluation. Five of the eight patients completing therapy had stable disease for at least 2 months (range, 2–6 months). Two patients had progressive disease at their first posttherapy assessment, both at 4 months.

RAS Mutation and Molecular Markers. Four patients' tumors were analyzable for RAS mutational status and P-Akt and EGFR expression. The P-Akt and EGFR expression were graded on a 0–3+ scale. Three of the four had K-*RAS* mutations (two at codon 12; one undetermined) with one of those tumors having an additional silent H-*RAS* mutation at codon 27. The tumor with no K-*RAS* mutation had 3+ EGFR and P-Akt expression. The tumor with mutated K-*RAS* at the undetermined codon had 1+ expression of both EGFR and P-Akt. The tumor with a K-*RAS* mutation at codon 12 but no H-*RAS* mutation had 3+ expression of both markers. The tumor from the patient who was the source of the PancM cell line had both H-*RAS* and K-*RAS* mutations. Expression of EGFR and P-Akt was not determined by immunohistochemical analysis in this cell line.

HDJ2 Farnesylation Studies. The effect of L-778,123 on HDJ2 prenylation in PBMCs was determined in 11 of the 12 patients. The mean percentages of nonfarnesylated HDJ2 as a function of treatment level and day of therapy are shown in Fig. 1. The mean interpatient percentage of nonfarnesylated HDJ2 increased from 3.9 ± 0.3% before infusion (days 1 and 22) to 18.6 ± 1.8% on the 8th day of infusion (days 8 and 29) for the 280 mg/m^2/day dose level compared with 3.8 ± 0.7% and 39.0 ± 4.2%, respectively, for the 560 mg/m^2/day dose.

***In vitro* Studies.** The PancM cell line expressing a K-*RAS* mutation and a silent H-*RAS* mutation was established from

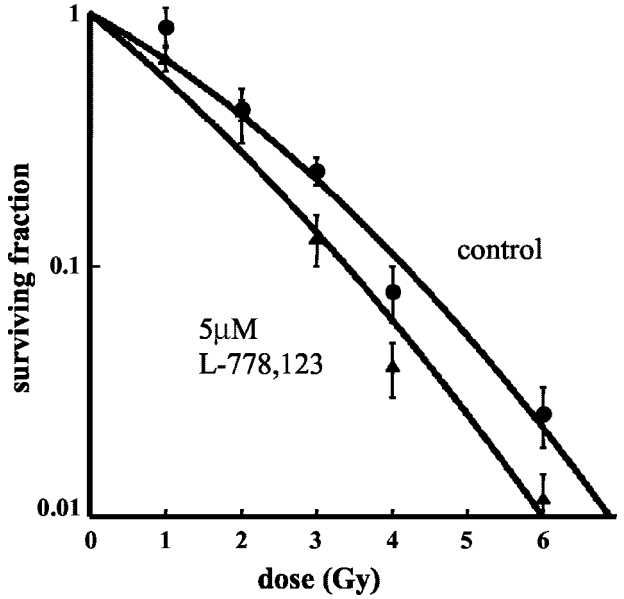

Fig. 2 Clonogenic survival of the PancM tumor cell line in the presence or absence of L-778,123. Log-phase cultures of PancM cells were plated for clonogenic survival in 100-mm dishes and exposed to radiation at the doses indicated in the presence (▲) or absence (●) of L-778,123. Inhibition with FTI was begun 24 h before and released 24 h after irradiation. Each point represents clonogenic survival at the indicated dose and error bars denote the SD from a minimum of 6 replicate plates. A 48-h exposure of PancM cells to L-778,123 resulted in significant radiosensitization compared with untreated controls.

the malignant ascites of a patient on therapy. Clonogenic cell survival was determined in the presence and absence of 5 μM L-778,123. As seen in Fig. 2, there was increased radiation sensitivity in the presence of the drug when compared with controls. Western blot analysis of the cultured cells revealed high P-Akt levels, which, along with mitogen-activated protein kinase phosphorylation, were reversed by L-778,123 treatment (data not shown).

DISCUSSION

This report describes a Phase I trial testing the feasibility of using the prenylation inhibitor L-778,123 in combination with radiotherapy in patients with locally advanced pancreatic cancer. The primary objective was to determine the tolerability and MTD of the therapeutic regimen. Patients with locally advanced pancreatic cancer were chosen for this study because they (*a*) face limited therapeutic options; (*b*) the therapeutic agent L-778,123 was designed to target cells with mutations in *RAS*, a very frequent finding in pancreatic cancer; and (*c*) chemoradiation is a standard therapeutic option for this population.

Two L-778,123 levels were evaluated in this trial, with no DLTs noted in the group treated with 280 mg/m^2/day. Two DLTs were observed in the patients treated with 560 mg/m^2/day consisting of grade 3 diarrhea and grade 3 GI hemorrhage associated with grade 3 thrombocytopenia and grade 4 neutropenia. Therefore, 280 mg/m^2/day given in weeks 1, 2, 4, and 5 should be considered the MTD in combination with upper abdominal radiotherapy. Other toxicities included grade 1 or 2 nausea and vomiting, hyperglycemia, dehydration, neutropenia, and electrolyte abnormalities. Compared with trials in which L-778,123 was used as a single agent, this trial found DLTs at a lower dosage when combined with radiotherapy (30, 31). One concern regarding the combination of new agents with radiotherapy is the possibility that an increase in severity, duration, or frequency of a radiation-associated toxicity might be observed. In our previous report of L-778,123 and radiation in NSCLC and HNC patients, no clear increase in these types of toxicities was observed (32). In this trial, diarrhea was observed in several patients including a relatively refractory case defined as a DLT in a patient treated at the second dose level. Despite its presence, the rate and severity of diarrhea seen with this combination therapy do not appear to represent a significant increase over what has been reported with high-dose radiotherapy alone (35). GI bleeding was observed in two patients. In one case, in conjunction with hematological toxicities, the hemorrhage constituted a DLT. The sites of both patients' GI bleeding were reviewed carefully because of the concern that they might represent a toxicity of combined modality therapy. Although radiation-induced GI ulceration and bleeding cannot be completely excluded in either case, it is reassuring that the site of the GI bleeding was from preexisting gastritis in the first patient and outside of the radiation field in the second. Viewed in light of the relatively frequent coagulation abnormalities seen in this study, care with regard to GI bleeding should be taken in future studies involving this class of drugs in combination with upper abdominal radiation.

Whereas response was studied as a secondary outcome, a clinical response was noted in only one of the eight evaluable patients. Furthermore, 4 of 12 patients enrolled on this study were unable to complete treatment, usually because of progressive disease. The cause of these disappointing results cannot be completely elucidated in this study, but pancreatic cancer is a disease known to be refractory to most standard therapies. One possible explanation for the lack of efficacy was that farnesylation was not altered by L-778,123 administration. Although blood L-778,123 levels were not measured in this study, the inhibition of HDJ2 farnesylation was evaluated as a surrogate for Ras farnesylation status. These studies showed farnesylation inhibition in what appears to have been a dose-dependent manner (Fig. 1). The results of the HDJ2 studies are encouraging, but conclusions regarding L-778,123 action cannot be made for a number of reasons. First, it is not clear that surrogate marker assessment is an accurate method to measure Ras farnesylation status (29). Secondly, the level of FTase inhibition required for radiosensitization is unknown.

The purpose of the *in vitro* experiments on the cell line (PancM) derived from a patient enrolled on this trial was to perform proof-of-principle studies of FTI radiosensitization. In these cells with H-*RAS* and K-*RAS* mutations, administration of L-778,123 led to radiosensitization (Fig. 2), as predicted by our previous preclinical work. Additional studies evaluating the effect of FTI administration on the Ras-phosphatidylinositol 3′-kinase-Akt pathway are being performed to further delineate the effects of FTI on signal transduction.

Despite extensive therapeutic research, patients with pancreatic cancer continue to face extremely poor response rates using standard treatment regimens. The factors underlying this

failure include the advanced tumor stage with which most patients present and the high rate of molecular abnormalities found in the tumors. In addition to a high prevalence of K-*RAS* mutations, overexpression of EGFR, Akt, caveolin-1, and Her2/neu are common findings (5, 7, 36–38). Prior studies have linked cellular radiation resistance to overexpression of several of these molecular marker abnormalities (26, 39, 40). Because these markers are associated with the Ras signaling pathway, inhibition of steps in the pathway might be expected to enhance radiation sensitivity.

Recent studies using FTIs as single-agent therapy or in combination with antineoplastic agents to treat solid tumors have shown only moderate disease-stabilizing effects (41–44). One explanation for these poor responses is that FTIs fail to inhibit the geranylgeranylation of K-Ras, allowing continued Ras transformation (45, 46). Because L-778,123 acts as an inhibitor of both FTase and geranylgeranyltransferase I, it might be expected to provide greater K-Ras inhibition than a FTI alone. However, a recent study showed that whereas the drug effectively inhibited both enzymes as measured on non-Ras proteins, it failed to inhibit K-Ras prenylation in PBMCs (29). Thus, although effective inhibition of farnesylation was shown on the surrogate marker HDJ2 in this study, Ras prenylation, and thus activation, may still have occurred.

It is not clear that inhibition of both H-Ras and K-Ras farnesylation, however, is required for radiosensitization (32). Mechanisms other than Ras inhibition for cellular radiosensitization with FTIs have been suggested. These include increased tumor oxygenation, cell cycle redistribution, and phosphatidylinositol 3′-kinase inhibition (47–49). The complexity of the molecular abnormalities in pancreatic cancer and the multiple signal transduction pathways activated in these tumors may account for the limited responses observed in the current study. The study highlights the need for further research to identify the molecular targets for the FTIs as well as markers that might predict response to therapy.

In this study, the MTD of L-778,123 in combination with upper abdominal radiation was determined to be 280 mg/m^2/day during weeks 1, 2, 4, and 5. It is noteworthy that none of the patients in either the pancreas or NSCLC/HNC cohorts experienced a DLT on this dose level (32). This suggests the tolerability of radiotherapy with concomitant prenylation inhibitors at doses somewhat lower than those used when administered as single agents (29). Because only two cohorts were used in this study, there may exist an untested intermediate dose of L-778,123 in combination with radiotherapy that also would have been tolerable. The development of L-778,123 was halted by the manufacturer as a result of QT_c prolongation. Whereas future clinical work with L-778,123 is not possible, there is continued enthusiasm for using other FTIs in combination with radiotherapy. Studies are currently under way exploring both this form of combination therapy and the molecular markers that underlie the relationship of FTIs to cellular radiosensitization.

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