

Phase I and Pharmacokinetic Study of Ecteinascidin 743 Administered as a 72-Hour Continuous Intravenous Infusion in Patients with Solid Malignancies¹

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ABSTRACT

Ecteinascidin 743 (ET-743) is a cytotoxic tetrahydroisoquinoline alkaloid that covalently binds to DNA in the minor groove. The *in vitro* chemosensitivity of cancer cells to ET-743 is markedly enhanced by prolonging the duration of exposure to the drug. A Phase I study of ET-743 given as a 72-h continuous i.v. infusion every 21 days was performed. Characteristics of the 21 adult patients with refractory solid tumors enrolled in the study were as follows: (a) 12 men; (b) 9 women; (c) median age, 59 years; (d) Eastern Cooperative Oncology Group performance status ≤ 1 , 20 patients; and (e) two prior regimens of chemotherapy, 7 patients. Dose limiting toxicity (DLT) was defined by typical criteria, except that grade 3 transaminitis did not constitute a DLT. There were no DLTs in the six patients evaluated at the first two dose levels of 600 and 900 $\mu\text{g}/\text{m}^2$. Reversible grade 4 transaminitis occurred in two of nine patients after treatment with the first cycle of therapy at the third dose level of 1200 $\mu\text{g}/\text{m}^2$. Another patient experienced grade 4 rhabdomyolysis, renal failure requiring hemodialysis, grade 4

neutropenia, and grade 3 thrombocytopenia during the second cycle of therapy with this dose. The maximum tolerated dose was 1200 $\mu\text{g}/\text{m}^2$, and an additional six patients were enrolled at an intermediate dose level of 1050 $\mu\text{g}/\text{m}^2$. This well-tolerated dose was established as the recommended Phase II dose. The disposition of ET-743 was distinctly biexponential, and a departure from linear pharmacokinetic behavior was evident at the 1200- $\mu\text{g}/\text{m}^2$ dose level. Pharmacokinetic parameters determined at 1050 $\mu\text{g}/\text{m}^2$ were (mean \pm SD): maximum plasma concentration, 318 \pm 147 pg/ml; initial disposition phase half-life, 9.0 \pm 10.3 min; terminal phase half-life, 69.0 \pm 56.7 h; and total plasma clearance, 28.4 \pm 22.5 liters/h/m². Prolonged systemic exposure to concentrations of the agent that are cytotoxic *in vitro* were achieved. Toxicity of the drug is clearly schedule-dependent, because increasing the duration of infusion from 3 or 24 h to 72 h results in decreased myelosuppression and comparable hepatotoxicity. Although there were no objective responses to therapy, clear evidence of antitumor activity was observed in a patient with epithelioid mesothelioma, as confirmed by positron emission tomography studies. A Phase II trial to assess the efficacy of ET-743 against this highly refractory neoplasm has been initiated on the basis of this observation. The therapeutically optimal administration schedule remains to be established, inasmuch as there have been indications of activity against a variety of tumors during Phase I studies when the drug was infused over times ranging from 1 to 72 h. Characterizing the pharmacokinetics of ET-743 during the course of Phase II trials and Phase I combination studies is recommended to assure that this promising new anticancer drug can be used with an acceptable margin of safety.

INTRODUCTION

The ecteinascidins are a family of naturally occurring tetrahydroisoquinoline alkaloids isolated from the Caribbean ascidian *Ecteinascidia turbinata* (1–3). Although several of these compounds exhibited exceptionally potent antitumor activity, the compound designated ET-743⁴ (Fig. 1) was ultimately selected for development as a clinical candidate because it was available in the greatest abundance from the natural source

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⁴ The abbreviations used are: ET-743, ecteinascidin 743; CTC, common toxicity criteria; LC₅₀, concentration producing 50% cell kill; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, glutamic-pyruvic transaminase; CT, computed tomography; PET, positron emission tomography; FDG, [¹⁸F]-2-fluoro-2-deoxy-D-glucose; HPLC, high-performance liquid chromatography; ESI-MS, electrospray ionization mass spectrometry; IS, internal standard; SV, switching valve.

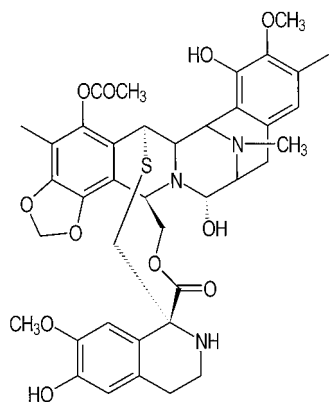


Fig. 1 Chemical structure of ET-743.

(4–7). When evaluated in the National Cancer Institute *in vitro* anticancer drug screen, ET-743 produced 50% cell death at low μM concentrations against cells in the colon, central nervous system, melanoma, renal, and non-small cell lung cancer panels and at low nM levels against breast, ovarian, and prostate cancer cell lines (6, 8). Promising growth-inhibitory activity has been demonstrated against a broad spectrum of human tumor xenografts implanted *s.c.* in nude mice after *i.v.* administration of the drug (6, 9, 10).

It appears that the cytotoxic effects of ET-743 result primarily from the selective alkylation of the N-2 amino group of a guanine residue in the minor groove of DNA by a mechanism that is analogous to that elucidated previously for the structurally related antitumor antibiotics saframycin, quinocarmycin, and naphthyridinomycin, as well as several other natural products (5, 11–15). However, the DNA sequence selectivity of ET-743 differs from any other compound known to alkylate DNA in this manner (13, 14). Moreover, the tetrahydroisoquinoline subunit joined to the main ring system by a 10-membered lactone bridge represents a key structural feature that distinguishes the ecteinascidins from other nonclassic DNA alkylators. This functionality protrudes perpendicularly outward from the molecule when bound to DNA and is believed to interfere with DNA-protein associations, thereby augmenting the selectivity and potency of the cytotoxic effects exhibited by the agent (14, 16). In addition, interactions of the drug with other molecular targets, such as the microtubule network and transcription factors, may contribute to its antiproliferative effects (17–19).

Consistent with a mechanism involving DNA modulation, ET-743 retards progression through the S phase of the cell cycle and promotes blockade at the G_2 -M boundary (6, 20). Exposure time represents an extremely important parameter for the chemosensitivity of cancer cells to ET-743. The drug proved to be substantially more toxic against human tumor cell lines when the duration of continuous exposure was extended from 1 h to periods ranging from 24 h to 3 days (6, 21). Longer exposure times also afforded a more favorable *in vitro* therapeutic index relative to toxicity against human hematopoietic progenitor cells (21). Furthermore, evaluation against primary tumor cells explanted from cancer patients using a clonogenic assay similarly indicated that both the efficacy and the potency of ET-743 were

markedly greater when specimens were subjected to prolonged continuous exposure, as compared with exposure for 1 h (22).

During the initial clinical development of ET-743, a series of Phase I trials in adults with solid tumors evaluated administration of the drug as a single *i.v.* infusion for periods of 1, 3, or 24 h as well as a schedule involving repeated daily treatment with a 1-h *i.v.* infusion for 5 consecutive days (23–26). The principal DLTs observed in these studies were neutropenia, thrombocytopenia, and fatigue. In addition, grade 3–4 elevations in serum transaminase levels occurred in 48–69% of the patients treated at the recommended Phase II doses but were not considered to be dose-limiting, because hepatotoxicity proved to be both reversible and noncumulative even after multiple courses of treatment. Antitumor responses were observed in patients with soft tissue sarcoma, melanoma, breast cancer, and osteosarcoma, and Phase II trials of the drug in 3- and 24-h infusion schedules are underway. The plausibility of enhancing the therapeutic index of the drug by prolonging the duration of systemic exposure, as suggested by preclinical studies, served as the rationale for undertaking an additional Phase I trial to assess the administration of ET-743 as a 72-h continuous *i.v.* infusion. The primary objectives of the study were to identify the DLTs, establish the MTD, and characterize the pharmacokinetic behavior of ET-743 when administered in this manner.

MATERIALS AND METHODS

Phase I Trial

Patient Selection. The study was restricted to patients with a histologically or cytologically confirmed solid tumor that was either refractory to conventional therapy or for which no standard treatment existed. Patients had to be at least 18 years of age with a minimum life expectancy of 3 months. The interval between major surgery or any other prior treatment of the malignancy and entry into this study had to be at least 6 weeks, with full recovery from the effects of the earlier intervention. Minimum eligibility requirements included the following: (a) Eastern Cooperative Oncology Group performance status ≤ 2 ; (b) neutrophil count $\geq 1,500/\mu\text{l}$; (c) platelet count $\geq 100,000/\mu\text{l}$; (d) hemoglobin ≥ 9.0 g/dl; (e) serum creatinine ≤ 2.0 mg/dl; (f) total bilirubin < 1.5 mg/dl; and (g) SGOT, SGPT, and alkaline phosphatase activities $\leq 3\times$ the upper limit of normal. Serum transaminase and alkaline phosphatase activities had to be $\leq 5\times$ the upper limit of normal for patients with documented liver metastases. The study excluded patients with: (a) evidence of a primary or metastatic lesion in the central nervous system; (b) baseline neurotoxicity greater than or equal to grade 2 (National Cancer Institute CTC); (c) prior bone marrow transplantation, required for stem cell support; (d) history of chronic active liver disease; or (e) pregnancy or breast feeding. A signed, written, informed consent document satisfying all federal and institutional requirements was obtained as a condition of patient registration.

Study Design. The study protocol was approved by the institutional Scientific Review Committee and the Human Protection Committee. Patients underwent a physical examination and performance status determination [an electrocardiogram and chest X-ray, a complete blood count with platelet and differential counts, coagulation tests (prothrombin time, partial throm-

boplastin time), a serum chemistry profile including γ -glutamyl transpeptidase activity, and urinalysis] within 14 days before initiating therapy. Treatment with any other approved or investigational antitumor drug, radiation, or growth factors, or acetaminophen was prohibited. The administration of antiemetic medications, with the exception of dexamethasone, was permitted at any time on an interventional or prophylactic basis.

The clinical dosage form of ET-743 was supplied by Pharma Mar, S. A. (Madrid, Spain) as a sterile lyophilized powder in glass vials containing 250 μg of the drug, 0.25 mmol of sodium phosphate, and 250 mg of mannitol. The drug was reconstituted by adding 5 ml of Sterile Water for Injection, USP, which afforded a clear buffered solution (pH 4). CADD programmable ambulatory infusion pumps (SIMS Deltec, St. Paul, MN) were used to deliver the drug through a central venous catheter without the use of an inline filter. All materials in the fluid path of the medication cassette reservoir and extension set were constructed from medical-grade polyvinyl chloride. The reconstituted drug solution was loaded into a 100-ml medication cassette reservoir and diluted further with Normal Saline for Injection, USP, such that the desired daily dose was delivered in a volume of 96 ml. A new medication cassette and an extension set containing a freshly prepared dosing solution was placed in the pump every 24 h.

ET-743 was administered as a 72 h continuous i.v. infusion at a starting dose of 600 $\mu\text{g}/\text{m}^2$ (200 $\mu\text{g}/\text{m}^2/\text{day}$). The dose was escalated at a constant increment of 300 $\mu\text{g}/\text{m}^2$ (100 $\mu\text{g}/\text{m}^2/\text{day}$). Cohorts of three patients were scheduled for entry at each dose level and treatment was repeated every 3 weeks as permitted by their condition. Escalation of the dose to the next higher level proceeded after all 3 patients received the first cycle of therapy with the preceding dose and had been observed for at least 21 days without evidence of a DLT, as defined below. An additional three patients were entered into a given dose level if a single patient experienced a DLT during the first cycle of therapy. Dose escalation proceeded in the absence of a DLT in these patients. The occurrence of a DLT in two patients from any cohort of three to six during the first cycle of therapy established the preceding dose level as the MTD. An additional six patients were enrolled at the MTD to better define toxicity and confirm tolerance.

Drug-related toxicities were evaluated during each cycle of therapy and graded according to the National Cancer Institute's CTC.⁵ A DLT was defined as any of the following events: (a) grade 4 neutropenia persisting for longer than 5 days; (b) grade 4 thrombocytopenia (platelet count $<25,000/\mu\text{l}$); (c) any drug-related nonhematological toxicity greater than or equal to grade 3 (except for grade 3 elevations in SGPT or SGOT that returned to baseline by day 21). Nausea, vomiting, alopecia, and hypersensitivity reactions were not considered to be DLTs. Hematological and clinical chemistry tests were obtained from all patients on days 1–5, 8, and 15 of every cycle of therapy. Serum chemistry evaluations were repeated daily until all parameters

returned to baseline values in the event of a change in any liver function test corresponding to grade 3 or 4 toxicity. This practice was amended during the course of the study to perform serum chemistry determinations every 48–72 h until all parameters returned to values associated with a toxicity of grade ≤ 1 .

Patients were scheduled to receive at least two courses of therapy with the same dose of ET-743 administered at intervals of 21 days. A patient history, physical examination, and evaluation of laboratory parameters were performed before each cycle of therapy to document that all eligibility criteria were satisfied. These assessments were also made whenever a patient was removed from the study. There was no limitation on the total number of cycles that a patient could receive. Inpatient dose escalation was not permitted. Patients experiencing toxicities that were not dose-limiting could be retreated at the same dose level upon full recovery. Treatment was discontinued upon the occurrence of a DLT or tumor progression.

Evaluation of Response. A baseline assessment of all measurable disease using any appropriate radiological technique was performed within 21 days before the first cycle of therapy. This included the acquisition of a CT scan and PET imaging of FDG uptake by the lesions identified by CT for all patients. Evaluations to assess therapeutic response by CT were performed after completing every second cycle of therapy until relapse. Additional FDG-PET scans were obtained during the second week of the first cycle of therapy and after every other course thereafter.

Tumor burden was calculated as the sum of the products of the longest perpendicular diameters of all measurable lesions. The duration of a response was measured from the date that the response was first recorded to the date of documented disease progression. Complete response was defined as the disappearance of all measurable disease, signs, symptoms, and biochemical changes related to the tumor. A reduction in tumor burden of $\geq 50\%$ constituted a partial response. Stable disease was defined as a $<50\%$ decrease in tumor burden or an increase $\leq 25\%$. In addition, for each of these classifications, the response or disease stabilization had to persist for a minimum of 4 weeks, during which time no new lesions were detected. Progressive disease was indicated by a $>25\%$ increase in tumor burden or the appearance of any new lesion.

Pharmacokinetic Studies. The plasma pharmacokinetics of ET-743 were characterized in all patients during administration of the first, second, and third cycles of therapy. Samples were collected before treatment and at 4, 6, 24, 48, and 72 h after starting the infusion for the first three cycles. Sampling to define the time course of drug decay from plasma was limited to the first treatment cycle, during which specimens were obtained at 0.25, 0.5, 1, 2, 3, 4, 6, 24, 48, 72 and 96 h after the end of the infusion. Blood specimens (8 ml) were acquired from an arm vein of the patient and collected in Vacutainer Brand tubes with freeze-dried sodium heparin anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Sample tubes were mixed by inversion and placed on ice until centrifuged ($2500 \times g$, 10 min, 4°C) within 15 min. Plasma was separated from the blood cells and stored at -70°C until assayed. The reading of a battery-powered digital timer was recorded when the infusion pump was started or stopped and when blood samples were collected.

⁵ The National Cancer Institute's CTC version 2.0 can be accessed from openly available information at the following internet address: <http://www.ctep.nci.nih.gov>.

Determination of ET-743 in Plasma

Synthesis of the IS. An IS structurally related to the drug, quinocarcinol octylamide, was synthesized by initially reducing quinocarcinoyl monochloride (National Cancer Institute, Bethesda, MD) with sodium borohydride in methanol (27). Quinocarcinol was isolated from the reaction mixture by solid phase extraction, redissolved in pyridine-methylene chloride (1:7, v/v), and condensed with octylamine using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride to activate the carboxylic acid moiety of the substrate. The crude product was purified by reversed-phase HPLC and solid phase extraction, affording quinocarcinol octylamide in high purity as a solution in methanol as established by LC-ESI-MS. The concentration of the IS in this solution was determined by measuring UV absorbance at 277 nm and assuming that its molar absorptivity was equivalent to that of quinocarcinoyl citrate in methanol (ϵ 1912 $\text{M}^{-1}\text{cm}^{-1}$).

Analytical Solutions. A stock solution of an analytical reference sample ET-743 (Pharma Mar S. A., Madrid, Spain) was prepared in methanol at a concentration of 0.10 mg/ml and stored at 0–5°C. A working solution of ET-743 (250 ng/ml) was prepared on a daily basis by diluting the stock solution with methanol-10 mM ammonium acetate buffer (pH 3.9; 1:1, v/v). Standard solutions were also made daily by serially diluting the ET-743 working solution with human donor plasma to concentrations of 500, 250, 100, 50, and 25 pg/ml. The working solution of the IS was made by diluting the solution of the purified synthetic material to a concentration of 7.4 ng/ml with methanol. Solutions of the IS were stored under refrigeration (0–5°C) and used for several months without evidence of degradation.

Sample Preparation. Frozen samples were thawed at ambient temperature, mixed on a vortex stirrer, and centrifuged for 10 min at $8000 \times g$. An aliquot of plasma (1000 μl), 6 μl of the IS working solution, and 1 ml of 0.2 M ammonium acetate buffer (pH 4.0) were combined in a borosilicate glass test tube and mixed by vortexing. This solution was applied onto a 3-ml (60-mg) Oasis HLB solid-phase extraction cartridge (Waters Corp., Milford, MA) that previously had been conditioned sequentially with 2 ml each of methanol, water, and 10 mM ammonium acetate buffer (pH 3.9). After washing with 3 ml of methanol-10 mM ammonium acetate buffer (pH 3.9; 1:3, v/v) and 3 ml of water, the cartridge was eluted with 1 ml of methanol. Moderate vacuum (≤ 15 in. Hg) was applied as necessary to facilitate flow through the cartridge, which was not permitted to exceed 1 ml/min, and to dry the stationary phase before the final desorption. The extract was evaporated to dryness under a stream of nitrogen at 40–45°C, reconstituted in 300 μl of methanol-10 mM ammonium acetate (pH 3.9; 1:1, v/v) and centrifuged ($8000 \times g$, 10 min). A 250- μl aliquot of the sample was injected into the chromatograph from a silanized borosilicate glass insert residing within an autosampler vial.

LC-ESI-MS Analysis. Analyses were performed using an HP 1100 Series LC/MSD system (Hewlett-Packard, Palo Alto, CA) consisting of a quaternary pump with a vacuum degasser, an isocratic pump, an autosampler fitted with a 500- μl sample loop, an automated six-position column SV, and a single-quadrupole mass selective detector equipped with an atmo-

spheric pressure ESI interface. The chromatographic instrumentation was configured for cleanup column back-flushing as follows: (a) flow from the quaternary pump was directed to the autosampler and then to SV port 6; (b) a 30-mm \times 4.6 mm I. D. Brownlee Spheri-5 phenyl (5 μm) cartridge column (Alltech Associates, Deerfield, IL) was connected between SV ports 2 (outlet) and 5 (inlet); (c) the isocratic pump was connected to SV port 3; (d) SV port 4 was connected to a 15 cm \times 3.9 mm I. D. Nova-Pak phenyl HPLC column (Waters Corp.) preceded by a Brownlee NewGuard phenyl guard cartridge; and (e) port 1 of the SV flowed to a waste reservoir. Both cartridge columns were preceded by 0.5- μm inline filters (Upchurch Scientific, Oak Harbor, WA).

Chromatography was performed at ambient temperature with both pumps operating at a flow rate of 1.0 ml/min. The SV was initially positioned to allow effluent from the cleanup column to flow to waste when injecting the sample. The sample was loaded onto the cleanup column using methanol-10 mM ammonium acetate buffer (pH 3.9; 75:25, v/v) as the eluent, which trapped the drug and IS on the stationary phase. After flushing the cleanup column for 5.5 min, the SV was rotated to bring the cleanup column into the flow path between the analytical column and the isocratic pump, delivering a mobile phase composed of methanol-9 mM ammonium acetate (pH 6.6; 75:25, v/v). The increase in pH of the mobile phase buffer induced elution of the drug and the IS from the cleanup column, with their transfer onto the analytical column being complete within 2 min. The SV was returned to its initial position at 7.5 min, whereupon the quaternary pump was programmed to wash the cleanup column with methanol-9 mM ammonium acetate (pH 6.6; 90:10, v/v) for 7.5 min and then re-equilibrate with the original eluent for the remaining 5 min of the 20-min run. Flow from the analytical column was directed into the nebulizer-assisted electrospray interface of the MSD without splitting. Operating parameters of the API-ES interface were as follows: (a) nebulizer pressure, 40 p.s.i.; (b) drying-gas, N_2 ; (c) drying-gas flow, 10 liters/min; (d) drying-gas temperature, 350°C; and (e) capillary voltage, 2000 V. The MSD was operated in the positive ionization mode with selected-ion monitoring. Ions corresponding to the base peaks in the API-ES mass spectra of ET-743 and the IS were measured at m/z 744.3 ($[\text{M}-\text{H}_2\text{O}+\text{H}]^+$) and m/z 444.2 ($[\text{M}+\text{H}]^+$), respectively, using a mass width of 0.07 units, a dwell time of 199 ms, and a fragmentor voltage of 150 V with the electron multiplier set at 1700 V. The chromatograms were integrated to provide peak areas using the data analysis functions of the HP ChemStation software, version A.06.03 (Hewlett-Packard).

Quantitation. All study samples were assayed together with a series of calibration standards on a daily basis. Standard curves were constructed by plotting the ET-743:IS chromatographic peak area ratio against the known concentration of ET-743. Linear least squares regression was performed using a weighting factor of $1/y$, without inclusion of the origin. Values of the slope and y-intercept for the best-fit line were used to calculate the analyte concentration in the study samples. Specimens exceeding the upper range of the standard curve were reassayed upon dilution with drug-free human plasma.

Pharmacokinetic Data Analysis. Sample times were calculated as the difference between the blood collection inter-

Table 1 Patient characteristics (n = 21)^a

Characteristic	Value
Age (yr)	
Median	59
Range	29–77
Sex (n) ^a	
Male	12
Female	9
Performance status (n) ^a	
0	5
1	15
2	1
Primary tumor site (n) ^a	
Colorectal	5
Leiomyosarcoma	4
Gastrointestinal stromal	2
Melanoma	2
Mesothelioma	2
Ovarian	2
Renal cell	2
Lung, non-small cell	1
Esophageal	1
Prior chemotherapy regimens (n) ^a	
0–2	14
≥3	7

^a n, number of patients.

val midpoint and the starting time of the infusion. The area under the plasma concentration-time profile from time zero to the end of the infusion [AUC(0-T)] was estimated by the linear/log trapezoidal algorithm using the WinNonlin version 1.1 software package (Scientific Consulting, Apex, NC). In addition, the complete plasma concentration-time profiles of the drug determined in individual patients during the first cycle of therapy were analyzed by nonlinear regression using WinNonlin, as described previously in detail (28). Values of the parameters corresponding to the equation that best described each plasma profile were used to calculate pharmacokinetic variables according to standard equations (29, 30). Mean values of pharmacokinetic variables were calculated as the geometric mean of the individual patient values (31). Standard deviations for the geometric mean values were estimated by the jackknife method (32). Parametric statistical tests of pharmacokinetic variables were performed after logarithmic transformation of the data (31, 33). Pearson sample correlation coefficients (*r*) were calculated to identify relationships between pharmacokinetic parameters and pretreatment laboratory values. The suggestion of a significant correlation, as indicated by $|r| \geq 0.4$, was substantiated by examining a scatter plot of the data and regression line, the *P* of the relationship, and the Spearman correlation coefficient (*r_s*). The maximum percent change in laboratory values observed during the first cycle of therapy was calculated as

$$\Delta\%_{\max} = \frac{|\text{pretreatment value} - \text{observed peak or nadir value}|}{\text{pretreatment value}} \times 100$$

for all tests, where the pretreatment value was the determination made immediately before dosing. Spearman correlations were performed to identify relationships between the maximum per-

Table 2 Doses of ET-743 and DLTs observed during the first cycle of therapy

Dose (μg/m ²)	Dose level	No. of patients	DLTs		
			Event	Grade	No. of patients
600	1	3	None		
900	2	3	None		
1050	4	6	Cardiac ^a	4	1
1200	3	9	Transaminitis	4	2
			Rhabdomyolysis	4	1 ^b
			Renal failure	4	1 ^b
			Neutropenia/fever	4	1 ^b

^a Supraventricular tachycardia.

^b Same patient during cycle 2 only.

cent change in laboratory values ($\Delta\%_{\max}$) and dose, maximum plasma concentration (C_{\max}) or the area under the plasma concentration-time curve from time zero to infinity (*AUC*) using $|r_s| \geq 0.4$ and *P* < 0.05 as the criteria for significance.

RESULTS

Patient Characteristics. A total of 21 patients were entered into the study between July 1998 and March 1999. Characteristics of these patients are listed in Table 1. The cohort included 12 men and 9 women with a median age of 59 years. All patients except for one had a performance status of 0 or 1, and seven patients had received three or more prior regimens of therapy for their malignancy.

Determination of the MTD. Dose escalation proceeded from 600 to 1200 μg/m² (Table 2). There was minimal toxicity at the first two dose levels, with the exception of a single episode of grade 3 transaminitis in a patient treated with 900 μg/m² ET-743. Two of nine patients experienced DLTs at the 1200-μg/m² dose level during the first cycle of therapy. Reversible grade 4 transaminitis occurred in these patients, one of whom received multiple cycles of therapy and had grade 4 transaminitis during the second course of treatment and grade 3 transaminitis during cycles 3 and 4. A third patient at the 1200-μg/m² dose level experienced grade 4 rhabdomyolysis during the second cycle of therapy and required hospitalization because of fever, neutropenia (grade 4 for 5 days), thrombocytopenia (grade 4), and acute renal failure. The patient fully recovered from all toxicities but required 6 weeks of hemodialysis before renal function improved. By study design, DLTs in the second cycle of therapy did not apply to the determination of the MTD. However, because of the occurrence of DLTs in two of nine patients during cycle 1 and severe rhabdomyolysis in a third patient during cycle 2, coupled with the suggestion of nonlinear pharmacokinetics at this dose level (see below), it was considered too risky to escalate the dose further. Therefore, the 1200-μg/m² dose level was designated the apparent MTD.

The study was then amended to evaluate a cohort of six patients at a dose of 1050 μg/m² to better define the toxicity and pharmacokinetics at an intermediate dose level. Aside from one occurrence of atrial fibrillation that was not believed to be drug-related, there were no other DLTs during the first cycle of therapy nor during any other cycle of therapy. Accordingly,

Table 3 Summary of clinical toxicities

Dose ($\mu\text{g}/\text{m}^2$)	No. of patients/cycles	Toxicity grade	No. of events observed (patients ^a /cycles)							
			Neutropenia	Thrombocytopenia	Transaminitis	Cardiac	Rhabdomyolysis	Renal	Fatigue	Nausea/ vomiting
600	3/8	1	0	0	1/3	0	0	1/1	2/3	0
		2	0	0	0	0	0	0	1/1	0
		3	0	0	0	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
900	3/6	1	0	0	0/1	0	0	1/2	2/4	2/3
		2	0	0	1/2	0	0	0	0	0
		3	0	0	1/1	0	0	0	0	0
		4	0	0	0	0	0	0	0	0
1050	6/10	1	0	0	2/3	0	0	0	2/3	3/3
		2	0	0	1/2	0	0	1/1	0	0
		3	0	0	3/5	0	0	0	0	1/1
		4	0	0	0	1/1	0	0	0	0
1200	9/22	1	1/2	2/3	0	0	0	0	5/13	5/7
		2	1/1	0	0/5	0	0	0	3/5	3/3
		3	1/1	0	6/9	0	0	0	1/3	0
		4	1/1	1/1	2/3	0	1/1	1/1	0	0

^a Maximum grade of toxicity experienced by each patient during all cycles of therapy.

1050 $\mu\text{g}/\text{m}^2$ was established as the recommended Phase II dose for the 72-h administration schedule.

Toxicities. Hematological and nonhematological toxicities observed during all cycles of therapy are summarized in Table 3. There were no clinically significant hematological toxicities (grade ≥ 1) at doses below 1200 $\mu\text{g}/\text{m}^2$. The patient treated with 1200 $\mu\text{g}/\text{m}^2$ ET-743 who experienced acute renal failure associated with severe rhabdomyolysis also had grade 4 neutropenia and grade 4 thrombocytopenia. Moreover, it was determined that this patient had the highest AUC (137.1 ng·h/ml) of ET-743 among all patients entered into the study. Only one other episode of grade 3 or higher hematological toxicity occurred at this dose level during 22 cycles of therapy.

Transaminitis was clearly the most common nonhematological toxicity observed. The frequency and severity of SGOT and SGPT elevations increased in a dose-dependent manner. At the first dose level (600 $\mu\text{g}/\text{m}^2$), three of eight (38%) cycles of therapy were complicated by grade 1 transaminitis, with grade 1–3 elevations occurring in four of six (66%) cycles at the second dose level (900 $\mu\text{g}/\text{m}^2$). At the 1200- $\mu\text{g}/\text{m}^2$ dose level, 9 of 22 cycles (41%) and 3 of 22 cycles (14%) were complicated by grade 3 and grade 4 transaminitis, respectively. Five of 10 cycles (50%) of therapy with the recommended Phase II dose of 1050 $\mu\text{g}/\text{m}^2$ were complicated by grade 3 transaminitis, and all six patients experienced at least grade 1 hepatic enzyme elevations. At each of the dose levels evaluated, the time course of transaminase elevations followed a predictable pattern, beginning 4–5 days after starting the 72-h infusion, peaking on days 7–9, and resolving by day 21 in 45 of 46 total administered cycles. Transaminitis was often accompanied by fatigue and right upper quadrant and/or shoulder discomfort that is characteristic of chemical hepatitis. There was no evidence of cumulative hepatotoxicity, because the degree of transaminitis in patients receiving multiple cycles of therapy was generally similar to that observed after the first dose.

Nonhematological toxicities other than hepatotoxicity were infrequently observed. Fatigue was a prominent symptom, being

at least moderate-to-severe in 5 of 21 patients (24%). One patient experienced new-onset atrial fibrillation that was not considered to be drug-related, but rather a consequence of underlying hypertensive heart disease. Nausea and vomiting were manageable, with only 4 patients experiencing grade ≥ 2 nausea/vomiting.

Antitumor Activity. Although no objective partial or complete responses were seen, evidence of antitumor activity was observed in two patients. A patient with malignant mesothelioma metastatic to the lungs, mediastinum, pleura, and peritoneum, who previously progressed on cisplatin, had a 41% reduction by CT scan in the size of measurable disease in the mediastinal lymph nodes and a pulmonary nodule, as well as a reduction in pleural and ascitic thickening. PET imaging performed after two cycles showed a 40% reduction in FDG uptake in the mediastinal lymph nodes. This patient had responding disease through four cycles of therapy before ultimately progressing. A patient with choroidal melanoma showed stable disease in the liver, although new mesenteric nodules were identified by CT and confirmed by percutaneous biopsy. PET imaging revealed a 60% decrease in FDG uptake within the liver lesions but failed to identify the new lesions.

Assay Validation. The analytical method used for measuring the concentration of ET-743 in plasma specimens acquired during the pharmacokinetic studies was developed specifically for use in this study. It was thoroughly validated according to currently recommended guidelines (34). Chromatograms of donor plasma and study specimens acquired from patients before, during, and after treatment with ET-743 showed no peaks, either of endogenous origin or attributable to a concurrently administered medication, that interfered with the detection of the drug or the IS. The limit of detection was 10 pg/ml (signal:noise ratio, 3.2), and the lower limit of quantitation was 25 pg/ml using a sample volume of 1.0 ml. Standard curves with ET-743 concentrations ranging from 25–500 pg/ml exhibited excellent linearity. In this concentration range, the grand mean (\pm SD) absolute recoveries of the drug and the IS were $95.8 \pm 13.1\%$

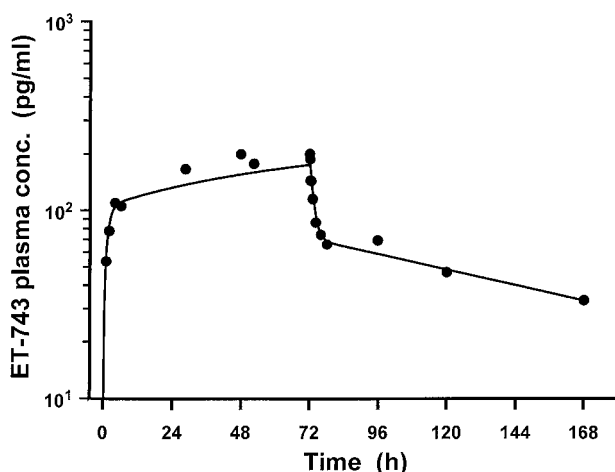


Fig. 2 Typical plasma concentration-time profile of ET-743 defined in an individual patient treated with a 1050 $\mu\text{g}/\text{m}^2$ dose of the drug given as a 72-h continuous i.v. infusion. Points (●) are the observed ET-743 plasma concentrations at each sample, and the solid line is the best-fit curve determined by nonlinear regression analysis of the data.

and $93.3 \pm 1.4\%$, respectively. Within-day accuracy of the assay was 82.7–119.2% at four concentration levels encompassing the standard curve, and the precision ranged from 4.7% at 500 pg/ml to 13.5% at 25 pg/ml ($n = 5$). Between-day accuracy and precision of the analytical method were assessed by analyzing the interpolated drug concentrations from a total of 33 standard curves run during a 12-week period. Mean values \pm SD of the regression parameters for these standard curves were: (a) slope, 0.00459 ± 0.00091 ; (b) y-intercept, -0.0183 ± 0.0277 ; and (c) correlation coefficient, 0.997 ± 0.003 . The grand mean between-day accuracy was $97.7 \pm 3.7\%$ (SD), and the precision ranged from 3.9% for the 500 pg/ml plasma standard to 13.7% at the 25 pg/ml limit of quantitation.

Pharmacokinetics. Complete plasma concentration-time profiles of ET-743 were defined during the first cycle of therapy in 20 of 21 patients. Pharmacokinetic data were not available from a single patient treated at the 1200 $\mu\text{g}/\text{m}^2$ dose level as a result of difficulties encountered in acquiring blood specimens attributable to poor venous access. A representative plasma profile determined in a patient that received a total dose of 1050 $\mu\text{g}/\text{m}^2$ ET-743 is shown in Fig. 2. Although plasma levels of the drug increased rapidly after the infusion was started, steady-state conditions were never truly achieved because the ET-743 plasma concentration continued to rise gradually throughout the 72-h infusion. The decline in plasma levels of the drug subsequent to the end of the infusion was distinctly biexponential in all 20 patients. Mean values of the pharmacokinetic parameters at each dose level calculated from the results of the nonlinear regression analysis of the individual patient data are summarized in Table 4.

As depicted in Fig. 3, mean values of the AUC determined during cycle 1 increased proportionately as the dose was escalated from 600 to 1050 $\mu\text{g}/\text{m}^2$ ($r = 0.993$). However, the AUC values of ET-743 for all eight patients evaluated at the 1200 $\mu\text{g}/\text{m}^2$ dose level were higher than predicted by extrapolation from the three lower dose levels, suggestive of a departure from

linear pharmacokinetic behavior at doses exceeding 1050 $\mu\text{g}/\text{m}^2$. This same trend was evident in the estimated C_{max} values of the drug, because the C_{max} and AUC were highly correlated ($r = 0.886$). The existence of dose-dependent pharmacokinetics was substantiated further by a statistically significant difference ($P = 0.027$; two-tailed t test) between the mean values of the total plasma clearance (CL) of ET-743 for the 12 patients treated with doses of 600–1050 $\mu\text{g}/\text{m}^2$ (29.6 ± 17.0 liters/h/ m^2) and the 8 patients at 1200 $\mu\text{g}/\text{m}^2$ (16.7 ± 8.1 liters/h/ m^2). The only other pharmacokinetic parameters that were significantly different between these two dosing groups were the initial disposition phase half-life ($t_{1/2,1}$; $P < 0.001$) and the apparent volume of the sampled compartment (V_1 ; $P = 0.047$; Table 4). Inspection of Fig. 3 provides the impression that interpatient variability in the AUC values may be greater for the 1200- $\mu\text{g}/\text{m}^2$ cohort than at the lower dose levels. However, this is not at all the case, because the magnitude of the coefficient of variation (CV) for the mean CL in patients evaluated at the 600–1050 $\mu\text{g}/\text{m}^2$ dose levels (57.5%) was very similar to that for the 1200- $\mu\text{g}/\text{m}^2$ cohort (48.4%). Plasma samples were obtained from a total of 16 patients during the 72-h i.v. infusion of the second course of ET-743. Paired values of the AUC(0-T) are depicted in Fig. 4. There was no significant difference between the dose-normalized AUC(0-T) for the first and second cycles of therapy with the drug ($P = 0.24$; paired two-sample, two-tailed t test).

At the recommended Phase II dose of 1050 $\mu\text{g}/\text{m}^2$, the C_{max} of ET-743 ranged from 180.5–646.8 pg/ml with a mean of 318.0 ± 147.3 pg/ml. In the 12 patients evaluated at the three lower doses (600, 900, and 1050 $\mu\text{g}/\text{m}^2$) conforming to linear pharmacokinetic behavior, the initial disposition phase was highly variable, with a half-life ranging from 2.0–59.9 min and a mean value of 8.9 ± 8.9 min. The terminal disposition phase was considerably longer, with a mean half-life of 61.0 ± 34.6 h. Because the ET-743 plasma concentration only decayed ~ 2 -fold during the relatively short time interval (1–2 h) from the end of the infusion until the onset of the terminal phase of drug disappearance, drug levels remained above the 25 pg/ml lower limit of quantitation of the assay for at least 96 h after the end of the infusion in the majority of patients, even at the starting dose. The similarity between the magnitude of the apparent biological half-life ($t_{1/2,z}$) and the mean residence time (MRT) of ET-743 calculated for these 12 patients, 52.3 ± 35.2 h, indicates that the slow terminal phase has a marked influence on the overall disposition of the drug. Whereas the mean V_1 (17.0 ± 19.1 liters/ m^2) was equivalent to 46% of body weight, the apparent volume of distribution at steady state (V_{ss}) was extremely large, being ~ 40 times greater than total body weight on average (1547 ± 800 liters/ m^2). This suggests that the drug has a much greater affinity for distribution into peripheral tissue compartments than for binding to plasma proteins. The only significant relationship between any ET-743 pharmacokinetic parameter and pretreatment serum chemistry values was a weak relationship between $t_{1/2,1}$ and total bilirubin ($r = -0.529$; $P = 0.017$; $r_s = -0.550$).

Pharmacokinetic-Pharmacodynamic Relationships. The only serum chemistry tests for which the $\Delta\%_{\text{max}}$ during the first cycle of therapy showed any significant correlation with the dose, C_{max} , or the AUC of ET-743 were those indicative of hepatotoxicity. Among these, the relationship between the max-

Table 4 Mean values of ET-743 pharmacokinetic parameters

Parameter	Dose level ($\mu\text{g}/\text{m}^2$)			
	600	900	1050	1200
<i>n</i>	3	3	6	8 ^b
C_{max} (pg/ml)	219.2 \pm 47.8 ^a	316.4 \pm 164.9	318.0 \pm 147.3	655.0 \pm 200.0
$t_{1/2,1}$ (min)	5.3 \pm 5.7	14.4 \pm 5.9	9.0 \pm 10.3	33.8 \pm 12.6 ^c
$t_{1/2,z}$ (h)	58.4 \pm 15.5	49.7 \pm 3.6	69.0 \pm 56.7	72.7 \pm 34.4
<i>MRT</i> (h)	47.0 \pm 9.6	34.1 \pm 1.7	68.4 \pm 62.6	64.2 \pm 44.2
<i>AUC</i> (ng·h/ml)	20.7 \pm 3.8	27.5 \pm 14.6	37.0 \pm 27.0	71.7 \pm 34.5
<i>CL</i> (l/h/m ²)	29.1 \pm 5.5	32.7 \pm 17.0	28.4 \pm 22.5	16.7 \pm 8.1 ^c
V_1 (l/m ²)	8.5 \pm 11.4	21.3 \pm 11.8	21.6 \pm 26.2	38.1 \pm 19.7 ^c
V_{ss} (l/m ²)	1365.8 \pm 516.7	1112.7 \pm 521.5	1940.5 \pm 1101.5	1074.5 \pm 427.0

^a Geometric mean \pm SD.

^b Pharmacokinetic data was not available from one patient because of poor venous access.

^c Significantly different from the mean of the parameter calculated for the 12 patients treated at the 600–1050- $\mu\text{g}/\text{m}^2$ dose levels (value in parentheses); $t_{1/2,1}$ (8.9 \pm 8.9 min), $P = 0.001$; *CL* (29.6 \pm 17.0 liters/h/m²), $P = 0.027$; V_1 (17.0 \pm 19.1 liters/m²), $P = 0.047$ (two-tailed *t*-test of log-transformed data).

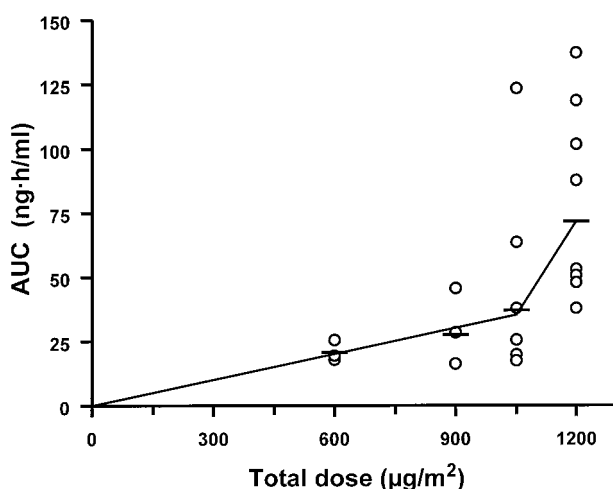


Fig. 3 Plot demonstrating the relationship between the *AUC* of ET-743 and the total dose administered during the first cycle of therapy. Points (○) are the observed values in individual patients; horizontal bars are the geometric mean values obtained for each group. The continuous solid lines terminating at 1050 $\mu\text{g}/\text{m}^2$ were generated by linear regression of the mean *AUC* values at the three lower dose levels.

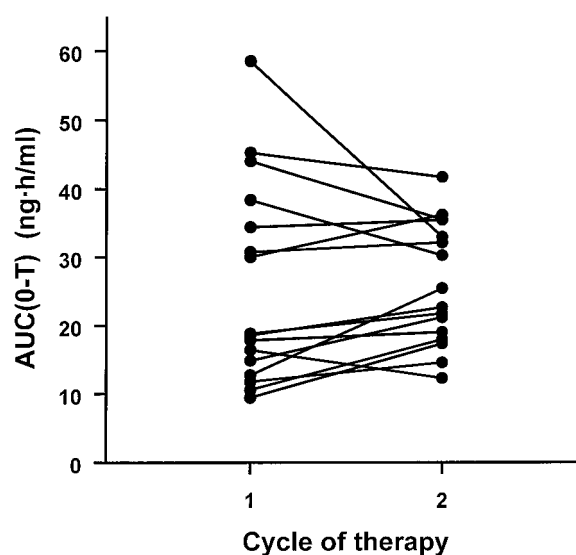


Fig. 4 Plot depicting the *AUC*(0-T) values in patients that received two cycles of therapy. Observed values in the same patient (●) are connected by line segments. There was no significant difference between the dose-normalized *AUC*(0-T) values for the first and second cycles ($P = 0.24$; paired two-sample, two-tailed *t* test).

imum percent increase in SGOT and *AUC* afforded the strongest correlation ($r_s = 0.803$; $P < 0.001$). This relationship is depicted in Fig. 5 together with the curve generated by fitting the sigmoid maximum effect model to the experimental data by nonlinear regression (35). In general, there was excellent agreement between the best-fit equation and the experimental data. Estimated values of the maximum effect, *AUC* corresponding to 50% of the maximum effect, and sigmoidicity parameter were 1138%, 88 ng·h/ml, and 2.0, respectively. Serum SGOT activity was substantially elevated in all patients after the administration of ET-743, even at the starting dose of the study. The smallest peak elevation was 20% above the pretreatment level. The *AUC*-effect relationship exhibited a steeply increasing region that was approximately log-linear, and a distinct plateau appears to have been achieved at the higher *AUC* values. These observations suggest that E_{max} and AUC_{50} were estimated with rea-

sonable confidence, although the absence of data at *AUC* values below the threshold producing a pharmacological effect imparts a relatively high level of uncertainty upon the estimate of the sigmoidicity parameter.

The only hematological parameter exhibiting a significant relationship was the maximum percent decrease in WBC, which was moderately correlated with dose ($r_s = 0.762$; $P < 0.001$), C_{max} ($r_s = 0.605$; $P < 0.01$) and *AUC* ($r_s = 0.622$; $P < 0.01$). Although severe neutropenia occurred in only two patients, there was nevertheless a distinct relationship between *AUC* and the relative decreases in WBC. A plateau in the magnitude of the effect was not evident and a simple linear equation ($r = 0.460$; $P < 0.001$) described the *AUC*-WBC effect profile as well or better than more complex pharmacodynamic models.

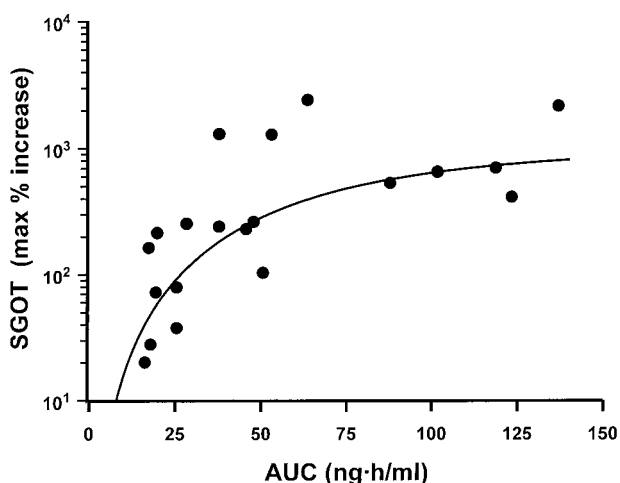


Fig. 5 Relationship between the maximum percentage increase in SGOT, expressed relative to pretreatment values, and the AUC of ET-743 during the first cycle of therapy. Peak SGOT elevations, depicted on a logarithmic scale because of the range of the values encountered, have been fitted to a sigmoidal E_{\max} equation (solid curve).

DISCUSSION

ET-743, a structurally unique cytotoxic alkaloid isolated from a marine organism, has been extensively evaluated in Phase I trials using several different administration schedules. It has shown *in vivo* activity against preclinical tumor models of human ovarian, breast, non-small cell lung, melanoma, sarcoma, and renal cancer (6, 9, 10). The molecular effects responsible for the cytotoxicity of the drug are not completely understood, although it has been conclusively established that the compound covalently binds to guanine N2 in the minor groove of DNA (13–15). The *in vitro* antiproliferative effects of ET-743 are clearly schedule-dependent (6, 21, 22). This would imply that the activity of ET-743 is cell cycle dependent, as supported by the observation that it blocks progression through the cell cycle at the late G_2 -M phase (6, 20). Increasing the duration of time that human tumor cell lines or primary human tumor cells are exposed to the drug results in enhanced sensitivity and a considerably greater degree of growth inhibition. Therefore, dosing regimens that provide sustained systemic exposure to the agent may be therapeutically advantageous.

Preclinical toxicology studies revealed that the principal DLTs of the compound were hematological and hepatic (6). Of particular concern, initial studies showed that the hepatotoxicity might not be completely reversible at very high doses in rodents, dogs, and nonhuman primates. Furthermore, female rats were considerably more sensitive to the hepatotoxic effects of ET-743 where these effects were both nonreversible and cumulative (6, 36). These observations are suggestive of a significant interspecies difference in the disposition or metabolism of the drug and indicated the importance of acquiring pharmacokinetic data during the early clinical evaluation of the drug in cancer patients. In the initial Phase I trials of ET-743, the drug was administered as a 1-h i.v. infusion given either once or on 5 consecutive days, with additional cycles of therapy administered every 21 days (23, 25). These studies confirmed that myelosup-

pression was the principal dose-limiting effect of ET-743 in cancer patients. In addition, grade 3–4 transaminitis, fatigue, peripheral vein phlebitis, nausea, emesis, and transient renal dysfunction were also observed.

In consideration of the time-dependence of the *in vitro* antiproliferative effects and schedule-dependent toxicity profiles observed in animals (6, 21, 22), Phase I trials of ET-743 given as a 24-h continuous i.v. infusion and, subsequently, as a 3-h infusion were undertaken in Europe (24, 26). The 24-h i.v. infusion provided sustained plasma levels that were well in excess of the *in vitro* LC_{50} against many human tumor cell lines. Myelosuppression was the DLT for both of these infusion schedules; however, it is important to note that the investigators did not consider grade 3 or 4 transaminitis to be dose-limiting. Reversible grade 3 and 4 transaminitis occurred in 69% and 48% of the patients treated at the recommended Phase II dose of the 3-h ($1650 \mu\text{g}/\text{m}^2$) and 24-h ($1500 \mu\text{g}/\text{m}^2$) infusion schedules, respectively. Despite its apparent reversibility, the hepatotoxicity was nevertheless concerning because of its severity. It remained to be seen whether a more prolonged exposure to ET-743 at plasma levels that were lower, but still exceeding the *in vitro* LC_{50} , would alter the toxicity profile. Therefore, the motivation for evaluating the administration of ET-743 by a 72-h continuous i.v. infusion schedule in the present study included: (a) exploiting the enhanced chemosensitivity exhibited by tumor cells upon prolonged exposure to the agent; (b) assessing whether the duration of time that plasma levels of the drug exceeded *in vitro* cytotoxic concentrations could be extended beyond that achieved with the shorter infusions schedules; and (c) evaluating the relationship between the toxicity (bone marrow and liver) and the pattern of systemic exposure provided by the administration schedule.

In this Phase I study, 21 adult patients with a variety of metastatic solid tumors were evaluated at four dose levels of ET-743, ranging from 600 to $1200 \mu\text{g}/\text{m}^2$, given as a 72-h i.v. infusion. The starting dose of $600 \mu\text{g}/\text{m}^2$ was the highest dose that did not produce any toxicities greater than grade 2 when given by 24-h i.v. infusion. Unlike the 24-h infusion schedule, for which there was no clinical evidence of hepatotoxicity in patients treated with $600 \mu\text{g}/\text{m}^2$ ET-743 and only one of eight cycles of $900 \mu\text{g}/\text{m}^2$ resulted in grade 3 elevations of hepatic enzyme levels in serum (5), transaminitis was apparent even at the starting dose upon giving the drug as a 72-h infusion. Transaminitis occurred with greater frequency, and it became progressively more severe as the dose was escalated. However, patients treated with multiple cycles of the drug did not experience worsening of the transaminitis with each successive cycle. Severe hematological toxicity was notably uncommon when ET-743 was administered as a 72-h infusion.

Dose-limiting nonhematological toxicities observed during the first cycle of treatment with $1200 \mu\text{g}/\text{m}^2$ ET-743 were reversible grade 4 transaminitis in two of nine patients. A third patient at this dose level experienced grade 4 rhabdomyolysis, grade 4 neutropenia, and grade 4 thrombocytopenia during the second cycle of therapy. Notably, this patient had the highest AUC (137.1 ng-h/ml) observed during this study. A careful review of all patients treated with ET-743 worldwide revealed that one other patient may have suffered drug-related rhabdomyolysis on the 1-h i.v. infusion daily $\times 5$ schedule (5). The

etiology of the rhabdomyolysis is unclear, and it was not observed during preclinical toxicology studies of the drug.

Characterizing the pharmacokinetic behavior of ET-743 has presented a particularly challenging problem, both in cancer patients and during preclinical studies, because this potent compound is tolerated at relatively low doses. The assay initially used to measure the drug in plasma during the Phase I studies was based upon HPLC with low-wavelength UV detection and had a lower limit of quantitation near 1 ng/ml (37). This level of sensitivity permitted ET-743 to be monitored for only ~30 min after a 1-h i.v. infusion (37). It was therefore apparent that a substantial improvement in sensitivity would be necessary to define the time course of ET-743 in plasma when given as a continuous i.v. infusion (38). This objective was realized by applying ESI-MS to detect ET-743 during HPLC. Two methods permitting the drug to be measured in plasma at concentrations in the 10–25-pg/ml range, one involving microbore HPLC coupled to a triple-quadrupole mass spectrometer (LC/ESI-MS/MS) and the other conventional HPLC with a single-quadrupole mass selective detector (LC/ESI-MS), were developed by Rosing *et al.* (38) and in our laboratory (39), respectively. These highly sensitive assays disclosed that the plasma concentration-time profile of ET-743 had a previously undetected disposition phase with a relatively long half-life and provided the basis for the pharmacokinetic sampling schedule in this study.

The plasma concentration of ET-743 declined in a distinctly biexponential manner after the end of the 72-h i.v. infusion and remained above the 25-pg/ml lower limit of quantitation of the assay at 96 h postinfusion in most patients. The initial disposition phase had a mean half-life of 8.9 min, but it was highly variable, with a 100% CV and a range of 2–60 min among the 12 patients treated with 600–1050 $\mu\text{g}/\text{m}^2$ ET-743. The mean half-life of the terminal disposition phase was 61.0 ± 34.6 h in this same group of patients. Consistent with the relatively long $t_{1/2,z}$, steady-state conditions were not achieved, as indicated by the continual increase in the ET-743 plasma concentration throughout the 72-h infusion. The mean $t_{1/2,z}$ of the drug in 22 patients treated with 1500 $\mu\text{g}/\text{m}^2$ given by 24-h infusion was 104.4 ± 36.6 h (40). When delivered as a 72-h i.v. infusion at doses of 600–1050 $\mu\text{g}/\text{m}^2$, the CL of ET-743 was independent of the administered dose with a mean value of 29.6 ± 17.0 liters/h/m². In comparison, the mean CL was 32.5 ± 14.0 liters/h/m² for the cohort of patients evaluated at the 1500- $\mu\text{g}/\text{m}^2$ dose level in the 24-h infusion study (40). The median CL in patients treated with 1650 $\mu\text{g}/\text{m}^2$ ET-743 given by 3-h infusion was 38.2 liters/h/m² (26). Thus, there was good agreement between the CL of ET-743 in patients treated with the 72-h and the shorter-term infusion schedules.

There was a departure from apparent linear pharmacokinetic behavior at the highest dose of 1200 $\mu\text{g}/\text{m}^2$ infused over 72 h, as demonstrated by a statistically significant decrease in CL to 16.7 ± 8.1 liters/h/m² ($P = 0.027$) relative to the combined group of patients treated with lower doses in this study. In contrast, when given as a 1-, 3-, or 24-h infusion, the plasma pharmacokinetics of the drug were described as being linear throughout the entire range of doses evaluated and also independent of the duration of infusion (41). The metabolism of ET-743 by rat liver preparations *in vitro* is mediated by cytochrome P-450 3A subfamily enzymes, with CYP3A4 being the

predominant isozyme catalyzing its biotransformation in a human liver panel (42, 43). However, the importance of hepatic metabolism and biliary excretion of unchanged drug as elimination pathways has not been quantitatively discerned in either humans or laboratory animals. Preliminary findings from this investigation indicate that only a very small fraction of the administered dosage, which appears to be <2%, is excreted in the urine as unchanged drug. Thus, it seems unlikely that the observed nonlinearity can be attributed to saturation of CYP3A4 or other hepatic enzymes involved in the metabolism of ET-743 because there was no evidence of decreased CL when comparable doses were given by infusions of shorter duration. In the absence of other explanations, it is entirely conceivable that the onset of liver damage at this dose level occurs early enough during the 72-h infusion to have an impact upon the subsequent elimination of a significant fraction of the administered drug. The hepatotoxic effects of ET-743 appear to be completely reversible, even in the six patients who received at least two cycles of the 1200- $\mu\text{g}/\text{m}^2$ dose, because there was no significant difference between the values of AUC(0-T) determined during the first and second cycles of therapy. Nevertheless, because of the suggestion that clinically significant alterations in the drug-eliminating capacity of the liver could occur during or shortly after treatment with ET-743, the potential for pharmacokinetic interactions should be recognized during initial clinical trials to combine it with other antineoplastic agents for which the liver represents an important eliminating organ.

The mean C_{max} of ET-743 provided by the 1050- $\mu\text{g}/\text{m}^2$ dose given as a 72-h i.v. infusion, 318 pg/ml (417 pM), is well above the *in vitro* LC₅₀ of the drug against human tumor cell lines in six disease categories of the National Cancer Institute antitumor screen (6). In comparison, mean values of the C_{max} at the recommended Phase II doses of 1650 $\mu\text{g}/\text{m}^2$ for the 3-h infusion (8.7 ng/ml)⁶ and 1500 $\mu\text{g}/\text{m}^2$ for the 24-h infusion (1.34 ng/ml) schedules were 27 and 4 times higher, respectively (40). Whereas severe transaminitis occurred frequently, even at the 600- $\mu\text{g}/\text{m}^2$ starting dose, and hepatotoxicity proved to be dose-limiting for the 72-h schedule, there was no evidence of hepatotoxicity when 600 $\mu\text{g}/\text{m}^2$ was infused over 24 h, and myelosuppression was the DLT for both the 3- and 24-h infusion schedules (24, 26). Furthermore, although the mean AUC for the 1200- $\mu\text{g}/\text{m}^2$ dose given by 72-h infusion (71.7 ng·h/ml) was greater than AUC values at the recommended Phase II doses for the 3-h (median, 43.2 ng·h/ml) and 24-h (mean, 57.6 ng·h/ml) schedules (26, 40), severe myelosuppression was rarely encountered. These observations suggest that the duration of time that the plasma concentration of ET-743 exceeds a threshold level is a more important determinant of hepatotoxicity than C_{max} or AUC. In addition, because myelosuppression is much more common at doses affording comparable AUC values for the 3- and 24-h infusions than for the 72-h infusion schedule (24, 26, 40, 41), it appears that the C_{max} is more closely associated with hematological toxicity than AUC.

In summary, this study has served to demonstrate that tolerated doses of ET-743 administered as a 72-h continuous i.v.

⁶ J. Jimeno, unpublished observations.

infusion provide prolonged systemic exposure to concentrations of the agent that are cytotoxic *in vitro*. The apparent MTD and recommended Phase II dose of ET-743 administered by this schedule are 1200 $\mu\text{g}/\text{m}^2$ and 1050 $\mu\text{g}/\text{m}^2$, respectively. Toxicity of the drug is clearly schedule-dependent, as prolonging the duration of infusion from 3 or 24 h to 72 h results in decreased myelosuppression and comparable hepatotoxicity. Although there were no objective responses to therapy, clear evidence of antitumor activity was observed in a patient with epithelioid mesothelioma, as documented by both CT scan and PET imaging. Tumor progression was minimal after six cycles, and the patient remained alive 1 year after stopping therapy. On the basis of this observation, a Phase II trial of ET-743 against mesothelioma was recently initiated at this institution. In other Phase I studies, partial responses have been noted in patients with soft tissue sarcomas, osteosarcomas, leiomyosarcoma, gastrointestinal stromal tumors, breast cancer, and melanoma (24, 26, 44). Phase II trials to evaluate the efficacy of the drug against many of these tumors are in progress. The therapeutically optimal administration schedule remains uncertain because there have been indications of activity when doses are infused over three hours or for one or more days. As with the anthracyclines, this may be an attribute of the prolonged terminal phase half-life of ET-743. Additional characterization of the population pharmacokinetics of ET-743 during Phase II trials and Phase I combination studies may provide extremely valuable information to assure that this promising new anticancer drug can be used with an acceptable margin of safety.

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