

# Pharmacokinetics and Pharmacodynamics of the Novel Marine-derived Anticancer Agent Ecteinascidin 743 in a Phase I Dose-finding Study<sup>1</sup>

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## ABSTRACT

Ecteinascidin (ET) 743 is an anticancer agent derived from the Caribbean tunicate *Ecteinascidia turbinata*. Preclinical studies revealed activity of ET-743 against different tumor types. A Phase I clinical trial was designed with ET-743 to identify the maximum tolerated dose and dose-limiting toxicities (DLTs). Furthermore, the pharmacokinetics of ET-743 and relationships with pharmacodynamics were evaluated. Adult patients with solid, resistant tumors received ET-743 as a 24-h i.v. infusion every 21 days. Blood samples were obtained during the first treatment course and in several consecutive courses. Noncompartmental pharmacokinetic analysis was performed. Relationships between pharmacokinetics and hepatic and hematological toxicities were explored. Fifty-two patients were treated at nine dose levels (50–1800  $\mu\text{g}/\text{m}^2$ ). The DLTs, neutropenia and thrombocytopenia, were experienced at 1800  $\mu\text{g}/\text{m}^2$ . Twenty-five patients were treated at the recommended Phase II dose of 1500  $\mu\text{g}/\text{m}^2$ . At this dose, the mean value  $\pm$  SD for total body clearance was  $59 \pm 31$  liters/h, and the mean  $t_{1/2}$  was  $89 \pm 41$  h. Pharmacokinetics were linear over the dose range tested. Prior exposure to ET-743 did not alter the pharmacokinetics in subsequent courses. The percentage of decrease in WBC count and absolute neutrophil count was correlated to the area under the plasma concentration versus time

curve (AUC). Hepatic toxicity, defined as rise in alanine aminotransferase and aspartate aminotransferase, increased with dose and AUC but was reversible and not dose limiting. In conclusion, ET-743 administered as a 24-h i.v. infusion at a dose of 1500  $\mu\text{g}/\text{m}^2$  is clinically feasible; severe thrombocytopenia and neutropenia are the DLTs.

## INTRODUCTION

A large proportion of the anticancer drugs currently used are derived from natural sources. They include the *Vinca* alkaloids, camptothecins, anthracyclins, and taxanes. During the past decade, the importance of marine organisms as a source of new materials has emerged and increased significantly (1, 2). Cytarabine was the first marine-derived anticancer drug marketed and is now an essential component in the treatment of acute myeloid leukemia. Several marine-derived compounds, e.g., bryostatin 1, dolastatins, and didemnin B, are now being clinically investigated as potential anticancer drugs (3–6). Didemnin B has shown activity against non-Hodgkin's lymphoma in Phase II clinical studies (7).

ET-743<sup>2</sup> (Fig. 1) is a representative example of a marine-derived anticancer product and was isolated from the Caribbean tunicate *Ecteinascidia turbinata*. More than 10 different ETs have been isolated; ET-743 was found to be a potent compound and appeared to be the most abundant in the tunicate (1). *In vitro* studies have identified activity of the drug against solid tumor cell lines including melanoma, non-small cell lung, ovarian, renal, prostate, and breast cancer (8, 9). Furthermore, *in vivo* experiments including several human xenograft models in mice demonstrated potent activity against non-small cell lung, ovarian, breast, renal, and melanoma tumors (1, 10, 11). Toxicity studies in rats, mice, dogs, and monkeys have shown hematological toxicity (anemia, leukopenia). Hepatic toxicity was observed as an increase in liver enzymes as well as evidence of cholestasis (1, 8).

The preclinical *in vivo* experiments with ET-743 revealed cytotoxic activity of the drug when administered at  $\mu\text{g}/\text{m}^2$  dosages, yielding nanomolar plasma concentrations. As a consequence, a very sensitive bioanalytical method is required to enable pharmacokinetic research. In our laboratory, an analytical system was developed that combines miniaturized LC with two mass analyzers (LC/MS/MS) with a lower limit of quantitation of 10 pg/ml (12, 13).

The mechanism of antitumor activity of ET-743 has not

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<sup>2</sup> The abbreviations used are: ET, ecteinascidin; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; RD, recommended dose; AUC, area under the plasma concentration versus time curve; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ANC, absolute neutrophil count; LC, liquid chromatography.

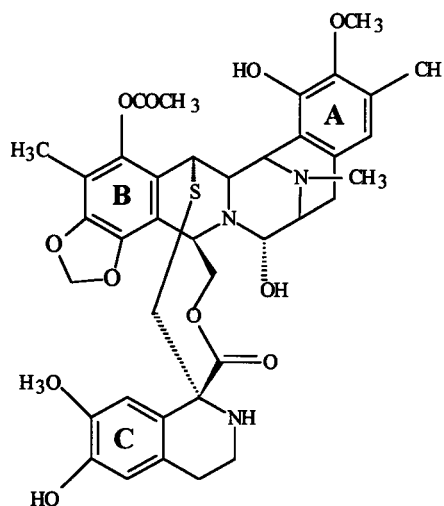


Fig. 1 The chemical structure of ET-743.

been completely elucidated yet, although it appears to be related to its ability to form covalent adducts at the N2 position of guanine in the minor groove of DNA (1, 14, 15). This binding interaction has been studied in more detail with nuclear magnetic resonance techniques, and it was reported that the units A and B and the carbinolamine moiety in ET-743 are responsible for the recognition of and bonding to DNA (14). Recently, it was described that by binding to the minor groove, ET-743 bends DNA toward the major groove (16). DNA-bound ET-743 appeared to modify the interaction between DNA and several transcription factors in which unit C of ET-743 is probably involved (15, 17, 18). Furthermore, ET-743 has been found to inhibit transcriptional activation of the *MDR1* gene by multiple inducers (19). Cytotoxic activity of ET-743 may also be caused by disorganization of the microtubule filaments in the cell (20). In addition, ET-743 has been found to block cell cycle progression in the late S and G<sub>2</sub>-M phases (1). A recent study also identified topoisomerase I (but not topoisomerase II) as a possible target of ET-743 (21).

Preclinical studies indicated an increased activity of ET-743 after long-term exposure (1, 8, 9). On the basis of these findings, a Phase I clinical study was designed and conducted with ET-743 administered as a 24-h infusion repeated every 3 weeks. The starting dose was based on mouse toxicology data, with a LD<sub>10</sub> of 200 μg/kg (600 μg/m<sup>2</sup>). The objectives of this trial were to determine the MTD and the DLTs and to propose a safe RD for additional Phase II investigation. Furthermore, the pharmacokinetics of ET-743 and relationships with the pharmacodynamics were evaluated at different dose levels.

## PATIENTS AND METHODS

**Patient Population.** Eligibility criteria included a histologically or cytologically confirmed diagnosis of a solid tumor not responding to any established form of treatment. Previous chemotherapy and/or radiotherapy was allowed provided that the last treatment was at least 4 weeks before study entry or 6 weeks in case of nitrosoureas, mitomycin C, and high-dose carboplatin. All pa-

Table 1 Patient characteristics

No. of patients	52
Male/Female	24/28
Median age, yr (range)	58 (19–74)
Primary tumor site	
Breast	9
Rectum	7
Colon	4
Gastrointestinal	9
Ovarian	6
Kidney	3
Pelvic	2
Unknown	2
Others	10

tients had acceptable bone marrow function (ANC,  $\geq 2.5 \times 10^9$ /liter; platelets,  $\geq 100 \times 10^9$ /liter; hemoglobin,  $\geq 10$  g/100 ml); adequate hepatic function (defined as serum bilirubin,  $< 25$  μM; transaminases and alkaline phosphatase,  $< 3 \times$  the upper normal limit, or  $< 5 \times$  the upper normal limit when attributable to liver metastases); and adequate renal function (serum creatinine  $\leq 120$  μM). Other eligibility criteria were a performance status of  $\leq 2$  on the Eastern Cooperative Oncology Group scale, age between 18 and 75 years, and a life expectancy of  $\geq 3$  months. The patients had to have a normal electrocardiogram and had to have recovered from any prior surgery. The study protocol was approved by the Medical Ethics Committee of the study center, and all of the patients gave written informed consent.

**DLTs.** All of the toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (22). DLTs were defined as any of the following events attributable to ET-743: any grade 4 toxicity (excluding alopecia and neutropenia); grade 3 toxicity (excluding emesis); grade 3 or 4 rise in AST, ALT, bilirubin, alkaline phosphatase, bile acids  $\geq 3 \times$  the upper normal limit, which have not recovered to grade 0 by day 21 and are still present at day 28; and grade 4 neutropenia lasting longer than 5 days or febrile neutropenia.

The dose at which at least two of three or three of six patients experienced DLTs was defined as the MTD. The next lower dose level below the MTD was defined as the RD for Phase II studies.

**Treatment Plan and Study Design.** ET-743 was supplied by Pharma Mar S.A. (Madrid, Spain) as a white-to-pale-yellow lyophilized powder in glass vials containing 250 μg of ET-743. The contents of a vial were reconstituted with 5 ml of water per injection, and the obtained solution was diluted in an additional 60 ml of a sterile 0.9% sodium chloride solution. ET-743 was administered i.v. via an electric syringe in 24 h through a central venous access.

The starting dose in this Phase I study was 50 μg/m<sup>2</sup>, administered as a 24-h i.v. infusion every 3 weeks. Dosage escalation in the second and subsequent dose levels was based on the safety profile observed at the previous level. Three new patients were entered at each dose level. If one patient exhibited DLTs, three additional patients had to be treated.

**Pharmacokinetics.** Serial blood samples (8 ml each) were collected in heparinized tubes at 15 time points: preinfusion; at 2, 6, and 23.5 h during the infusion; and at 5, 10, and 15 min and at 0.5, 1, 2, 4, 6, 9, 12, and 24 h after the end of the

Table 2 Pharmacokinetic parameters (mean  $\pm$  SD) of ET-743 after a 24-h i.v. infusion during course 1

Dose level ( $\mu\text{g}/\text{m}^2$ )	<i>n</i>	$AUC_{\text{inf}}$ (h $\cdot$ ng/ml)	$C_{\text{max}}$ (ng/ml)	$t_{1/2}$ (h)	$Cl_{\text{tot}}$ (liters/h)	$V_{\text{SS}}$ (liters)
50	3	1.2 ( $\pm$ 0.67)	0.061 ( $\pm$ 0.043)	10 ( $\pm$ 9.4)	79 ( $\pm$ 39)	570 ( $\pm$ 210)
100	3	2.1 ( $\pm$ 1.7)	0.078 ( $\pm$ 0.051)	20 ( $\pm$ 26)	134 ( $\pm$ 81)	790 ( $\pm$ 680)
200	3	3.8 ( $\pm$ 1.5)	0.19 ( $\pm$ 0.08)	25 ( $\pm$ 19)	100 ( $\pm$ 42)	1700 ( $\pm$ 1600)
400	3	39 ( $\pm$ 45)	0.76 ( $\pm$ 0.49)	81 ( $\pm$ 110)	38 ( $\pm$ 26)	1100 ( $\pm$ 740)
600	3	12 ( $\pm$ 4.8)	0.56 ( $\pm$ 0.22)	22 ( $\pm$ 12)	96 ( $\pm$ 57)	2100 ( $\pm$ 2300)
900	3	36 ( $\pm$ 16)	0.95 ( $\pm$ 0.20)	64 ( $\pm$ 58)	53 ( $\pm$ 20)	2200 ( $\pm$ 1600)
1200	5	32 ( $\pm$ 13)	1.4 ( $\pm$ 0.65)	24 ( $\pm$ 6.8)	79 ( $\pm$ 35)	1200 ( $\pm$ 580)
1500	25	55 ( $\pm$ 25)	1.8 ( $\pm$ 1.1)	89 ( $\pm$ 41)	59 ( $\pm$ 31)	3900 ( $\pm$ 1900)
1800	4	50 ( $\pm$ 17)	1.9 ( $\pm$ 0.58)	94 ( $\pm$ 28)	64 ( $\pm$ 23)	5300 ( $\pm$ 3200)

Table 3 Pharmacokinetic parameters (mean  $\pm$  SD) of ET-743 after a 24 i.v. infusion during multiple courses

Dose level ( $\mu\text{g}/\text{m}^2$ )	Course	<i>n</i>	$AUC_{\text{inf}}$ (h $\cdot$ ng/mL)	$C_{\text{max}}$ (ng/ml)	$t_{1/2}$ (h)	$Cl_{\text{tot}}$ (liters/h)	$V_{\text{SS}}$ (liters)
1200	1	5	32 ( $\pm$ 13)	1.4 ( $\pm$ 0.65)	24 ( $\pm$ 6.8)	79 ( $\pm$ 35)	1200 ( $\pm$ 580)
1200	2	4	37 ( $\pm$ 23)	0.98 ( $\pm$ 0.51)	26 ( $\pm$ 12)	72 ( $\pm$ 31)	1300 ( $\pm$ 540)
1500	1	25	55 ( $\pm$ 25)	1.8 ( $\pm$ 1.1)	89 ( $\pm$ 41)	59 ( $\pm$ 31)	3900 ( $\pm$ 1900)
1500	2	20	52 ( $\pm$ 39)	1.5 ( $\pm$ 1.1)	74 ( $\pm$ 59)	72 ( $\pm$ 44)	3900 ( $\pm$ 3500)
1500	5	4	32 ( $\pm$ 9.0)	0.99 ( $\pm$ 0.23)	45 ( $\pm$ 36)	81 ( $\pm$ 20)	2300 ( $\pm$ 1400)
1800	1	4	50 ( $\pm$ 17)	1.9 ( $\pm$ 0.58)	94 ( $\pm$ 28)	64 ( $\pm$ 23)	5300 ( $\pm$ 3200)
1800	2	4	64 ( $\pm$ 23)	1.1 ( $\pm$ 0.069)	127 ( $\pm$ 47)	48 ( $\pm$ 12)	5500 ( $\pm$ 1400)

infusion. Plasma was obtained by immediate centrifugation of the samples (15 min, 4000  $\times$  g). The clear supernatant was transferred into a polypropylene tube and stored at  $-20^\circ$ . At the lower dose levels (50–900  $\mu\text{g}/\text{m}^2$ ), only the first courses of treatment were pharmacokinetically monitored; at higher dose levels, the second and fifth courses were also sampled in the majority of the patients. Because ET-743 displays a long terminal elimination half-life ( $t_{1/2}$ ), the sampling schedule was extended at the 1500- and 1800- $\mu\text{g}/\text{m}^2$  levels to obtain the complete terminal part of the curve; blood samples were then collected up to 300 h after the end of administration.

For the bioanalysis of ET-743, a method was used that couples miniaturized LC to an electrospray sample inlet (ESI) and two quadrupole mass analyzers (LC/ESI/MS/MS; Ref. 13). Solid-phase extraction on cyano columns was used as a sample pretreatment procedure. Dry residues were redissolved in reconstitution solvent containing the internal standard ET-729, and an aliquot was injected into the LC system. A methanol-water (75:25, v/v) mixture containing 5 mM ammonium acetate and 4% (v/v) formic acid was pumped through a C18 column with a flow rate of 200  $\mu\text{L}/\text{min}$ . The column outlet was directly connected to the sample inlet without splitting. Ions were created at atmospheric pressure and were transferred to a quadrupole mass spectrometer. The method was validated over a range of 10–2500 pg/ml using 500  $\mu\text{L}$  of plasma. The assay was linear over this range and provided within-day and between-day precision of  $<9.3\%$  for all of the quality control samples. The average accuracy at four different concentrations ranged from 97 to 103%.

The pharmacokinetic parameters were calculated by applying a noncompartmental analysis using the pharmacokinetic WinNonlin program (Standard Edition Version 3.0, 1999). The maximum drug concentration ( $C_{\text{max}}$ ) was derived directly from the experimental data. The terminal rate constant ( $k$ ) was estimated by log-linear regression analysis of the terminal phase of the plasma concentration versus time curve. The area under the plasma con-

centration-time curve ( $AUC_{\text{inf}}$ ) was determined using the log-linear trapezoidal method with extrapolation to infinity using the terminal rate constant  $k$  ( $C_{\text{last}}/k$ , where  $C_{\text{last}}$  is the last measured analyte concentration). The  $t_{1/2}$  was calculated from the equation  $0.693/k$ ; total plasma clearance ( $Cl_{\text{tot}}$ ) was determined by dividing the total administered dose ( $\mu\text{g}$ ) by the  $AUC_{\text{inf}}$ . The apparent volume of distribution at steady state ( $V_{\text{SS}}$ ) was calculated as  $V_{\text{SS}} = Cl_{\text{tot}} \times MRT_{\text{inf}}$ , where  $MRT_{\text{inf}}$  is the mean residence time, which is determined as  $MRT_{\text{inf}} = (AUMC_{\text{inf}}/AUC_{\text{inf}}) - (1/2 \times \text{duration of infusion})$ , where  $AUMC_{\text{inf}}$  is the area under the first moment curve with extrapolation to infinity.

**Statistical Analyses.** Pharmacokinetic linearity between dose and  $AUC_{\text{inf}}$  of the first course was evaluated using linear regression analysis. Differences in pharmacokinetic parameters between the first, second, and fifth treatment cycles were evaluated using the paired  $t$  test. Baseline demographic and biochemical patient characteristics were examined as possible determinants of the pharmacokinetic parameters. Relationships were investigated between pharmacokinetic parameters and age, weight, body surface area, serum creatinine, creatinine clearance, and total protein, using Pearson's correlation coefficient and linear regression. The effect of the presence of liver metastases at the start of treatment and the effect of gender on pharmacokinetic parameters were evaluated using the independent samples  $t$  test.

Statistical analyses were performed with SPSS (Statistical Product and Service Solutions, version 6.1 for Windows, 1994). All of the tests for significance were two-tailed, and the level of significance ( $P$ ) was set at 0.05.

**Pharmacodynamics.** The relationships between the dose or pharmacokinetic parameter ( $AUC_{\text{inf}}$  or  $C_{\text{max}}$ ) and pharmacodynamics were explored using data of the first courses at all of the dose levels. For the evaluation of hepatic toxicities, the National Cancer Institute Common Toxicity Criteria grading system for AST, ALT, and alkaline phosphatase was used. Hematological

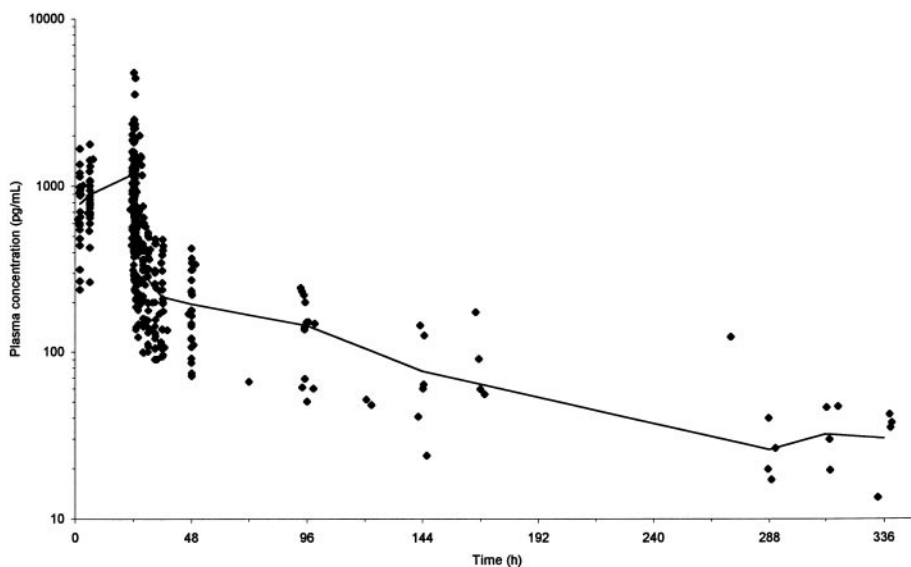
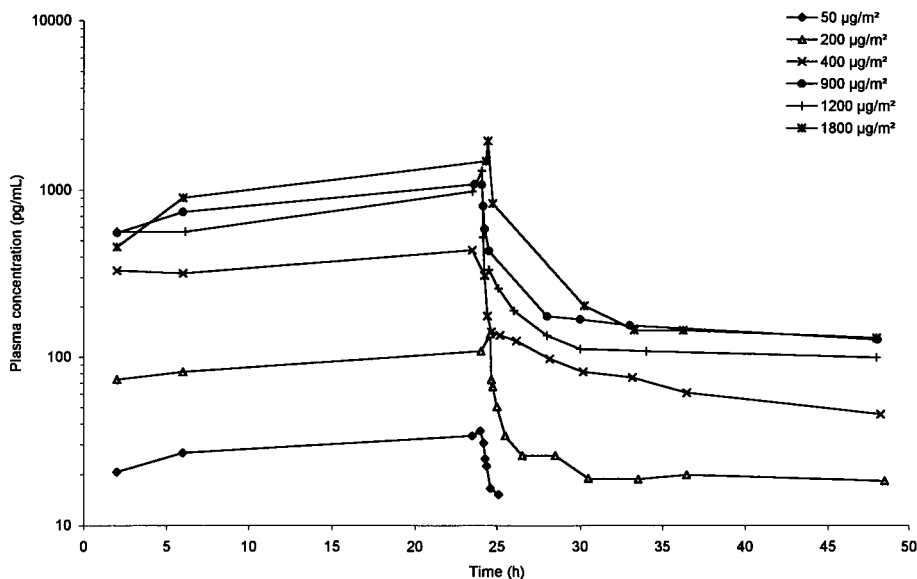


Fig. 2 Plasma concentration versus time for all of the patients treated with 1500  $\mu\text{g}/\text{m}^2$  ET-743 during the first course of treatment. Solid line, mean plasma concentrations.

Fig. 3 Typical plasma concentration versus time profiles at different dose levels of ET-743.  $\blacklozenge$ , 50  $\mu\text{g}/\text{m}^2$ ;  $\triangle$ , 200  $\mu\text{g}/\text{m}^2$ ;  $\times$ , 400  $\mu\text{g}/\text{m}^2$ ;  $\bullet$ , 900  $\mu\text{g}/\text{m}^2$ ;  $+$ , 1200  $\mu\text{g}/\text{m}^2$ ;  $*$ , 1800  $\mu\text{g}/\text{m}^2$ .



toxicities were evaluated using the percentage of decrease in WBC, ANC, platelets, and hemoglobin. The percentage of decrease was calculated using the following equation:

Percentage of decrease

$$= \frac{\text{Pretreatment value} - \text{value of the nadir}}{\text{Pretreatment value}} \times 100\% \quad (\text{A})$$

The relationship between hematological toxicities and pharmacokinetics were fit to a sigmoidal maximum effect model ( $E_{\text{max}}$ ; Ref. 23) using SPSS. The sigmoidal  $E_{\text{max}}$  model is given by

$$\text{Percentage of decrease} = \frac{E_{\text{max}} \cdot (P)^\gamma}{(P_{50})^\gamma + (P)^\gamma} \quad (\text{B})$$

In this equation,  $E_{\text{max}}$  denotes the maximum effect, and  $P$  is the pharmacokinetic parameter of interest,  $P_{50}$  represents the value of  $P$  that results in 50% of the  $E_{\text{max}}$ , and  $\gamma$  is the Hill coefficient, which describes the sigmoidity of the curve.

## RESULTS

**Patients and Treatment.** A total of 52 patients was enrolled in this study. Patient characteristics are listed in Table 1. The following dose levels were evaluated: 50, 100, 200, 400, 600, 900, 1200, 1500, and 1800  $\mu\text{g}/\text{m}^2$ , administered as a 24-h i.v. infusion every 21 days. At the doses 50 to 900  $\mu\text{g}/\text{m}^2$  three patients were treated at each level during multiple courses; pharmacokinetic profiles were obtained during the first course. Five patients were given 1200  $\mu\text{g}/\text{m}^2$ , of which nine courses were pharmacokineti-

Fig. 4  $AUC_{inf}$  versus dose level during the first course.

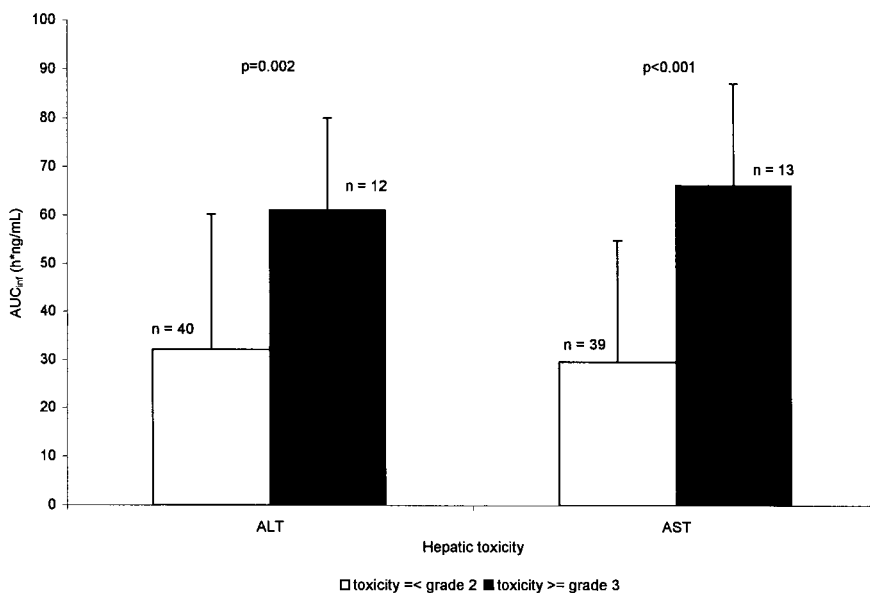
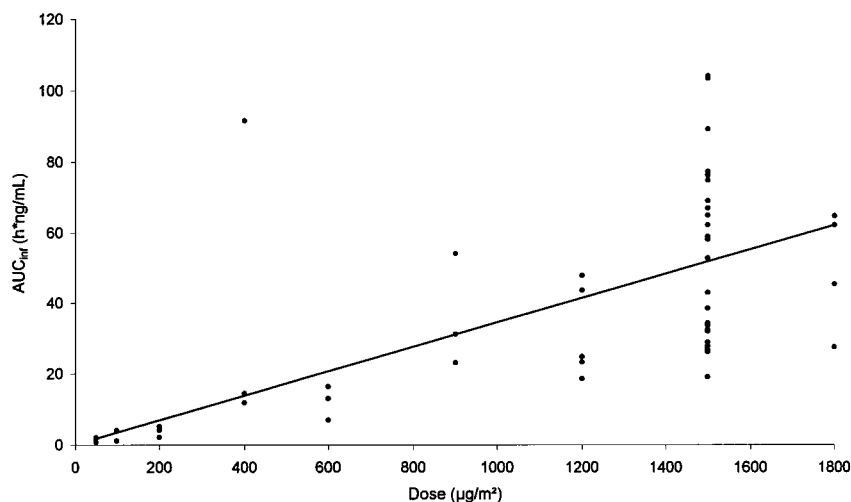


Fig. 5 Mean values for the  $AUC_{inf} \pm SD$  observed in patients experiencing grade 2 or lower ALT/AST elevation ( $\square$ ) or grade 3 or higher ALT/AST elevation ( $\blacksquare$ ). Left, ALT; right, AST.

cally monitored (five first and four second courses). Four patients received  $1500 \mu\text{g}/\text{m}^2$ ; blood samples were obtained in 10 courses (four first and second courses, two fifth courses). Four patients were treated with  $1800 \mu\text{g}/\text{m}^2$ ; in all four patients, the first and second courses were pharmacokinetically monitored. In one patient, samples were collected during the sixth course; these data were not taken into account for additional analyses. One patient started at a dose of  $1800 \mu\text{g}/\text{m}^2$ , and two courses were sampled; during the fifth course, which was also monitored, the patient received  $1500 \mu\text{g}/\text{m}^2$ . The results of the fifth cycle were not included in additional evaluations.

At a dose of  $1800 \mu\text{g}/\text{m}^2$  DLTs were experienced; hence this dose was identified as the MTD. DLTs were severe thrombocytopenia and neutropenia. Hepatic toxicity, defined as increase in AST and ALT, occurred, although it was not dose limiting, and seemed to be transient and not cumulative. The RD was chosen at one dose level below the MTD at  $1500 \mu\text{g}/\text{m}^2$ .

Another 21 patients were then recruited to be treated at the RD to better define the safety profile before commencing a Phase II program. Blood samples were obtained in 40 courses (21 first, 16 second, and 3 fifth courses).

**Pharmacokinetics.** Pharmacokinetic parameters of ET-743 at all of the dose levels and during multiple courses were calculated; mean values ( $\pm SD$ ) are presented in Tables 2 and 3. Plasma concentrations of all of the patients treated at  $1500 \mu\text{g}/\text{m}^2$  are shown in Fig. 2. Typical plasma concentration versus time profiles at different dose levels of ET-743 are shown in Fig. 3.

The  $AUC_{inf}$  increased linearly with increasing dose ( $r$ , 0.65;  $P < 0.001$ ; Fig. 4). Dose-normalized  $AUC_{inf}$  was independent of dose ( $r$ ,  $<0.001$ ;  $P = 0.477$ ), indicating linear pharmacokinetics of ET-743 over the dose range and schedule studied. Interpatient variability, however, was considerable (the coefficient of variation for  $AUC_{inf}$  at  $1500 \mu\text{g}/\text{m}^2$  was 45%), resulting in overlap in  $AUC_{inf}$  between different dose levels.



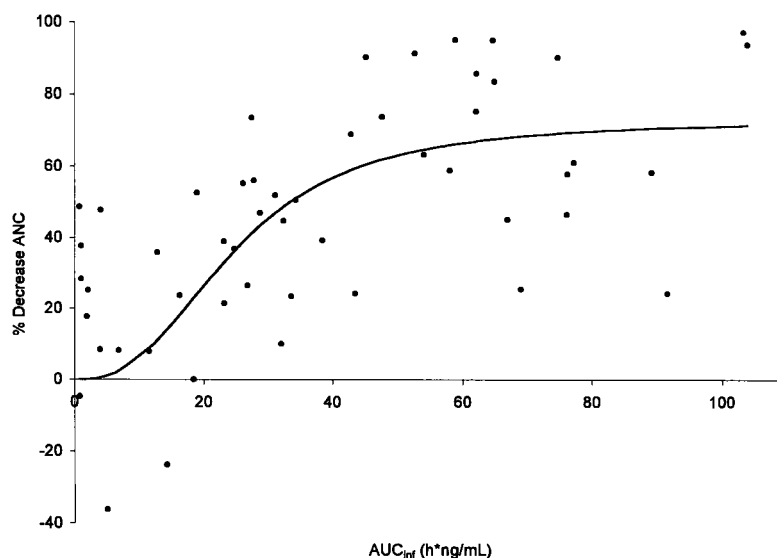


Fig. 6 The percentage of decrease in ANC versus  $AUC_{inf}$ . Solid line, fit of the data to sigmoidal  $E_{max}$  model.

Inpatient variability in  $AUC_{inf}$  between the first and the second course was relatively small (coefficient of variation at  $1500 \mu\text{g}/\text{m}^2$  was 28%,  $n = 20$ ).

The influence of prior exposure to ET-743 on the pharmacokinetics of subsequent cycles could be evaluated at the three highest dose levels (Table 3). Complete pharmacokinetic profiles were obtained in patients receiving  $1500 \mu\text{g}/\text{m}^2$  during the first course ( $n = 25$ ), the second course ( $n = 20$ ), and the fifth course ( $n = 4$ ). Pharmacokinetic parameters did not differ significantly between the first and the second courses, indicating that previous treatment with ET-743 had no effect on its pharmacokinetics in the subsequent course. However, a statistically significant increase in total body clearance ( $Cl_{tot}$ ) was observed between the first and fifth courses ( $P = 0.04$ ) and the second and the fifth courses ( $P = 0.01$ ).

At both dose levels,  $1200$  and  $1800 \mu\text{g}/\text{m}^2$ , pharmacokinetic studies were performed in four patients during the first and the second courses. No statistically significant differences in any pharmacokinetic parameter could be observed between the first and second courses at both levels, which is consistent with the results at  $1500 \mu\text{g}/\text{m}^2$ .

Patient characteristics were investigated as determinants of interpatient pharmacokinetic variability. No significant relations could be identified between tested pharmacokinetic parameters ( $AUC_{inf}$ ,  $Cl_{tot}$ ,  $V_{ss}$ ,  $t_{1/2}$ ,  $C_{max}$ ) and age, weight, body surface area, gender, serum creatinine, creatinine clearance, and total protein levels. The presence of liver metastases did not seem to alter total body clearance of ET-743; the data revealed no significant difference in  $Cl_{tot}$  between patients with ( $n = 20$ ) or without liver metastases ( $n = 32$ ;  $P = 0.657$ ).

#### Pharmacokinetic-Pharmacodynamic Relationships.

Transaminase levels increased with dose,  $AUC_{inf}$  and  $C_{max}$  during the first course; although less obvious, an increase in alkaline phosphatase was also noted. Patients experiencing a grade 3 or 4 increase in AST and ALT during the first course showed significantly higher values for the  $AUC_{inf}$  than did patients with grade 2 or lower increase in AST and ALT ( $P < 0.001$  for AST and  $P = 0.002$  for ALT; Fig. 5). For alkaline phosphatase this relationship was not significant.

Pharmacokinetic parameters ( $AUC_{inf}$ ,  $C_{max}$ ) of all of the patients during the first course of treatment and the corresponding dosages [both dose ( $\mu\text{g}/\text{m}^2$ ) and total dose ( $\mu\text{g}$ )] were plotted against the percentage of decrease in ANC, WBCs, platelets, and hemoglobin. The data revealed no significant correlations between dosage or pharmacokinetic parameters and the percentage of decrease in hemoglobin. The percentage of decrease in platelets was significantly correlated to  $AUC_{inf}$  ( $r = 0.41$ ;  $P = 0.002$ ); however, the correlations with dosage and  $C_{max}$  were not statistically significant. The percentage of decrease in WBC count was significantly correlated to dose, total dosage,  $AUC_{inf}$ , and  $C_{max}$  ( $r = 0.56$ ,  $P < 0.001$ ;  $r = 0.54$ ,  $P < 0.001$ ;  $r = 0.63$ ,  $P < 0.001$ ; and  $r = 0.45$ ,  $P = 0.01$ , respectively). Correlations between the percentage of decrease in ANC and dose, total dosage,  $AUC_{inf}$ , and  $C_{max}$  were also significant ( $r = 0.63$ ,  $P < 0.001$ ;  $r = 0.62$ ,  $P < 0.001$ ;  $r = 0.63$ ,  $P < 0.001$ ; and  $r = 0.45$ ,  $P = 0.01$ , respectively). The relation between the percentage of decrease in ANC, which was dose limiting, and the  $AUC_{inf}$  could be described using the sigmoidal  $E_{max}$  model (Fig. 6). The estimate of the maximum decrease in ANC ( $E_{max}$ ) in this study was 73.1%. The  $AUC_{inf}$  that resulted in 50% of the maximum effect was  $24.9$  (h\*ng/ml), and  $\gamma$  was 2.63.

## DISCUSSION

In this Phase I study, the pharmacokinetic profile of ET-743 was evaluated in a total of 86 courses at different dose levels and multiple cycles; this is the first report on ET-743 pharmacokinetics. The RD for Phase II studies was identified at  $1500 \mu\text{g}/\text{m}^2$ . At this dose, a total of 25 patients have been treated to obtain more population safety and kinetic data before large-scale Phase II evaluation in different tumor types. The recommended Phase II dose appeared to be safe, and indications for antitumor activity have been noted (24). DLTs were severe neutropenia and thrombocytopenia. Although not dose limiting, hepatic toxicity was observed as well and appeared to be transient and not cumulative. This toxicological profile is consistent with the findings in preclinical experiments (1, 8).

At the dose range tested, the pharmacokinetics of ET-743 were found to be linear. Considerable interpatient variability in the pharmacokinetics of ET-743 was observed at all of the dose levels evaluated. The possible influence of patient characteristics on this variability was explored. No obvious correlations could, however, be identified between pharmacokinetic parameters and patient demographics or renal and liver function at study entry. Of note, no relationship existed between BSA and pharmacokinetic parameters. The presence of liver metastases at study entry did not alter the  $Cl_{tot}$  of ET-743.

Deviating mean pharmacokinetic parameters with large interpatient variability were observed at a dose of 400  $\mu\text{g}/\text{m}^2$ ; the mean value for  $AUC_{inf}$  was 39 h·ng/ml, although the median value was 14 h·ng/ml. In this treatment group, one patient displayed a long  $t_{1/2}$  of the drug ( $t_{1/2} = 203$  h), resulting in an extremely high value for the  $AUC_{inf}$  (91.6 h·ng/ml). The reason for the high  $AUC_{inf}$  value is unclear. Interestingly, this did not result in severe toxicity for this patient. At a dose of 1500  $\mu\text{g}/\text{m}^2$ , one patient experienced grade 3 asthenia and grade 4 neutropenia and thrombocytopenia with epistaxis during the second course of treatment. This serious adverse event was most likely caused by the deviating high value for the  $AUC_{inf}$  (205 h·ng/ml) that the patient displayed during this course. The value for the  $AUC_{inf}$  during the first course (60 h·ng/ml) was within the normal range.

At the lower dose levels (50–100  $\mu\text{g}/\text{m}^2$ ), plasma concentrations in the terminal part of the curve were less than the limit of quantitation (10 pg/ml) and, thus, could not be quantified, although a very sensitive analytical method was developed (13). As a result, the  $t_{1/2}$  of ET-743 at these doses was underestimated and markedly shorter than in the other treatment groups.

Initially, the first course of treatment of each patient was pharmacokinetically monitored. However, as the study progressed, it appeared that the AST/ALT elevations, which were grade 3 in the majority of the patients during the first two courses of treatment, diminished in the later courses. To investigate whether this rather unexpected phenomenon could be explained by changes in the pharmacokinetics of ET-743, consecutive courses were sampled as well at the three highest dose levels (1200, 1500, and 1800  $\mu\text{g}/\text{m}^2$ ). At all three dose levels, pharmacokinetic parameters did not differ significantly between the first and second course, which implied that repeated administration does not markedly alter the kinetics of the drug. However, at a dose of 1500  $\mu\text{g}/\text{m}^2$ , a significant increase in  $Cl_{tot}$  was observed between the first and fifth courses and between the second and fifth courses. This is most likely caused by the fact that, during the fifth courses, fewer blood samples were taken from the terminal part of the curve than in previous courses. Consequently, by using noncompartmental pharmacokinetic analysis, values of  $Cl_{tot}$  are overestimated. Therefore, it cannot be concluded that the decrease in the severity of ALT/AST elevation in the later courses of treatment is related to a change in the pharmacokinetic profile of ET-743.

Hepatic toxicity of ET-743 increased with dose and  $AUC_{inf}$ , both in frequency and severity, although it was not dose limiting because it had recovered to grade 0 before the next dose. Patients who experienced increase in AST and ALT of grade 3 or higher showed significantly higher values for  $AUC_{inf}$  than those with AST and ALT elevations of grade 2 or lower. The mechanism of the reversible hepatotoxicity is under current investigation. In preclinical studies, this phenomenon was also observed (1).

The present study revealed significant correlations between the percentage of decrease in ANC and WBC and dose and pharmacokinetic parameters  $AUC_{inf}$  and  $C_{max}$ . Furthermore, the percentage of decrease in platelets was significantly correlated to  $AUC_{inf}$ . In general, the highest values for correlation coefficients were seen with the percentage of decrease in ANC. The relation between the dose-limiting ANC and  $AUC_{inf}$  could be adequately described using a sigmoidal  $E_{max}$  model, although considerable variability was observed. Therefore, other models (e.g., linear or logarithmic) could have been applied as well, but the sigmoidal  $E_{max}$  model was most obvious on biological grounds because it may be assumed that the percentage of decrease ranges from 0 to 100%. The data in this study approximately cover this range (Fig. 6). Three patients experienced a probably insignificant increase in ANC after administration of ET-743. All three patients had relatively low baseline levels at study entry, and they were treated at the lower dose levels (100 and 400  $\mu\text{g}/\text{m}^2$ ). Correlations between percentage of decrease in ANC and dose (either in  $\mu\text{g}$  or  $\mu\text{g}/\text{m}^2$ ) were significant as well. However, at the RD of 1500  $\mu\text{g}/\text{m}^2$ , large variability (10–100%) in the percentage of decrease in ANC was observed. The range of variability in the percentage of decrease was reduced (60–80%) when it was related to the value for  $AUC_{inf}$  at this dose (55 h·ng/ml on average), and, therefore,  $AUC_{inf}$  was considered a better predictor for neutropenia than dose.

To reduce the interpatient variability in  $AUC_{inf}$ , a benefit may be obtained from individual adaptation of the administered dose (25). Because no relationships were found between pharmacokinetic parameters and patient demographics, dose individualization cannot be used in the first course. For consecutive cycles, however, the dose may be adapted on the basis of the pharmacokinetics in the previous cycle, because inpatient variability was relatively small.

Data on the metabolism of ET-743 are scarce, and, thus far, the metabolic fate has not yet been elucidated. *In vitro* incubation experiments with ET-743 and rat and human hepatic microsomes have shown a time-dependent decrease of the drug concentration (26), although metabolic products could not be identified. These experiments have also generated indications that demethylation may occur, because formaldehyde is formed during the incubation experiments. However, *N*-desmethyl ET-743 (ET-729) could not be detected. This is supported by the finding of Rosing *et al.*, (13) that there was no ET-729 in the plasma of treated patients, which therefore allows this compound to be used as internal standard for the assay. ET-743 metabolic clearance after incubation with male rat liver microsomes appeared to be substantially higher than with female microsomal preparations (26), which is probably caused by the male predominance of the responsible enzyme (CYP3A2) in this species. These differences in metabolism may contribute to the higher sensitivity of female rats for ET-743, as was seen in *in vivo* toxicity studies. It is unlikely that this difference in rate of metabolism between male and female rats could also be observed in humans because human cytochrome P450 enzymes do not exhibit such gender differences (26). In fact, in the present clinical study, this is confirmed because no significant difference was found for  $Cl_{tot}$  or  $t_{1/2}$  between male and female patients ( $P = 0.14$  and  $0.23$ , respectively).

The starting dose in this Phase I study was 50  $\mu\text{g}/\text{m}^2$ , which

was approximately one-tenth of the LD<sub>10</sub> (or MTD) found in mice (200 µg/kg or 600 µg/m<sup>2</sup>, respectively). Dose escalation was based on the modified Fibonacci procedure. A pharmacologically guided escalation, as proposed by Collins *et al.* (27) was not applicable for this study because several requirements for the safe use of an accelerated escalation were not met. The administration of the drug in patients (24-h infusion) was prolonged compared with that in the preclinical studies (bolus injection). Furthermore, the mode of action of ET-743 has not been completely elucidated, and more work is needed to clarify the metabolism.

In preclinical studies, ET-743 showed potent cytotoxic activity at nanomolar concentrations (8–10). The plasma concentrations reached in patients at the RD were of the same order of magnitude. Although differences in the schedule of administration and the possible differences in sensitivity to the drug may hinder extrapolation to patients, these are promising results for the Phase II efficacy studies.

The presented Phase I trial indicates that ET-743 administered as a 24-h i.v. infusion every 3 weeks is well tolerated; the DLTs were neutropenia and thrombocytopenia. At the dose range tested, we observed linear pharmacokinetics and considerable interpatient variability. The RD was identified at 1500 µg/m<sup>2</sup>, and Phase II studies have already commenced at this dose and schedule in different tumor types, including advanced soft tissue sarcomas, melanoma, and breast and renal cancer.

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