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## Clinical report

# Clinical pharmacology of the novel marine-derived anticancer agent Ecteinascidin 743 administered as a 1- and 3-h infusion in a phase I study

**Charlotte van Kesteren,[1] Chris Twelves,[2] Angela Bowman,[6] Klaas Hoekman,[4] Luis López-Lázaro,[3] José Jimeno,[3] Cecilia Guzman,[3] Ron AA Mathôt,[1] Andrew Simpson,[2] Jan B Vermorken,[5] John Smyth,[6] Jan HM Schellens,[1] Michel JX Hillebrand,[1] Hilde Rosing[1] and Jos H Beijnen[1]**

[1]Department of Pharmacy and Pharmacology, The Netherlands Cancer Institute/Slotervaart Hospital, Amsterdam, The Netherlands. [2]Cancer Research Campaign, Department of Medical Oncology, Beatson Oncology Centre, Glasgow, UK. [3]Pharma Mar, Clinical R & D, 28760 Tres Cantos (Madrid), Spain. [4]Free University Hospital, Amsterdam, The Netherlands. [5]Antwerp University Hospital, Edegem, Belgium. [6]Western General Hospital, Edinburgh, UK.

**Ecteinascidin 743 (ET-743) is an anticancer agent derived from the Caribbean tunicate *Ecteinascidia turbinata*. In the present article, the pharmacokinetics and pharmacodynamics of ET-743 are described within a phase I study. Forty patients with solid tumors initially received ET-743 as a 1-h i.v. infusion every 21 days at nine dose levels (50–1100 $\mu$g/m$^2$). The maximal tolerated dose (MTD) was 1100 $\mu$g/m$^2$, with thrombocytopenia and fatigue as dose-limiting toxicities (DLTs). As this MTD was substantially lower than in parallel phase I studies, dose escalation continued using a prolonged, 3-h infusion. Thirty-two patients were entered at five dose levels (1000–1800 $\mu$g/m$^2$). The MTD was 1800 $\mu$g/m$^2$ with pancytopenia and fatigue as DLTs. The recommended phase II dose was 1650 $\mu$g/m$^2$ given over 3 h at which 12 patients were treated. Pharmacokinetic monitoring was performed for both treatment schedules. Non-compartmental pharmacokinetic parameters at the recommended dose with the 3-h infusion were (mean value±SD): clearance 87±30 l/h and mean elimination half-life 26±7 h. Pharmacokinetics were linear at the dose range tested with this schedule. The percentage decrease in platelets, white blood cells and neutrophils correlated with the area under the plasma concentration versus time curve (AUC), dose and maximal plasma concentration ($C_{max}$). Hepatic toxicity increased with dose, AUC and $C_{max}$. Administration of 1650 $\mu$g/m$^2$ ET-743 over 3 h seemed clinically feasible; pharmacokinetics were linear with this schedule. Hepatic and hematological toxicities correlated with exposure to ET-743. [© 2002 Lippincott Williams & Wilkins.]**

Correspondence to Corresponding author. Ch van Kesteren, Department of Pharmacy and Pharmacology, The Netherlands Cancer Institute/Slotervaart Hospital, Louwesweg 6, 1066 EC Amsterdam, The Netherlands.
Tel: (+31) 20 5124657; Fax: (+31) 20 5124753;
E-mail: apcks@slz.nl

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## Introduction

During the past decade, the importance of marine organisms as a source of new anticancer agents has emerged and increased significantly.[1–3] Several marine-derived compounds, e.g. bryostatin 1, dolastatins and aplidin, are now being clinically investigated as potential anticancer drugs.[3]

Ecteinascidin 743 (ET-743, Figure 1), a tetrahydroisoquinoline alkaloid, is a novel marine derived anticancer product and was isolated from the Caribbean tunicate *Ecteinascidia turbinata.*[4] More than 10 different ecteinascidins have been isolated; ET-743 was found potent and appeared to be the most abundant of these compounds in the tunicate.[1] *In vitro* studies have identified activity of ET-743 against solid tumor cell lines including melanoma, non-small cell lung, ovarian, renal, prostate and breast cancer.[5] 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.[1,6,7] Toxicity studies in rats, mice, dogs and monkeys have all shown hematological toxicity (anemia, leukopenia).[8] Hepatic toxicity was observed as an increase in liver enzymes as well as cholestasis.[1]

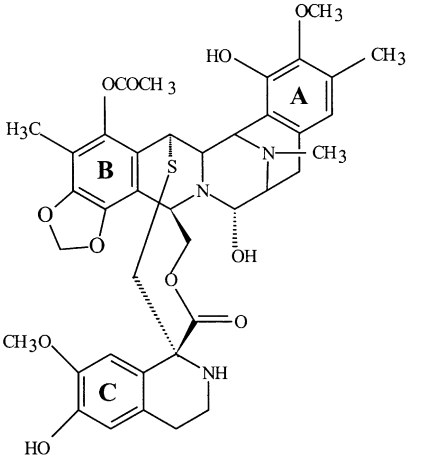


**Figure 1.** The chemical structure of ET-743.

The preclinical *in vivo* experiments with ET-743 revealed cytotoxic activity of the drug when administered at $\mu g/m^2$ dosages, yielding nanomolar plasma concentrations. As a consequence, a very sensitive bio-analytical method is required in order to enable pharmacokinetic research. An analytical system was developed in our laboratory, which combines miniaturized liquid chromatography with two mass analyzers (LC/MS/MS) with a lower limit of quantification of 0.01 ng/ml.[9,10]

The mechanism of antitumor activity of ET-743 has not been completely elucidated yet, although it appears to be related to its ability to form covalent adducts at the $N_2$ position of guanine in the minor groove of DNA.[1,11,12] Detailed studies using NMR techniques revealed that the units A and B, and the carbinolamine moiety in ET-743 (Figure 1) are responsible for the recognition of and binding to DNA.[12] Recently, it was described that by binding to the minor groove, ET-743 bends DNA toward the major groove.[13] 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.[12,14,15] Furthermore, ET-743 has been found to inhibit transcriptional activation of the MDR1 gene.[16] ET-743 appeared to block cell cycle progression in the late S and $G_2$/M phases,[1] and hypersensitivity for ET-743 of cells in the $G_1$ phase has been demonstrated.[17] In addition, a recent study suggested that topoisomerase I is not a critical target for the cytotoxic activity of ET-743.[18] A phase I clinical program was designed which included the evaluation of several infusion schedules for ET-743.[19–22] Recently, the pharmacokinetic profile of ET-743 administered as a 24-h infusion[19] and as a 72-h infusion[21] were described. As part of the phase I program, a clinical study was conducted with ET-743 administered as a 1-h infusion, repeated every 3 weeks. The starting dose in this trial was based on mouse toxicology data with a $LD_{10}$ of 200 $\mu g/kg$ (600 $\mu g/m^2$). The objectives were to determine the maximum tolerated dose (MTD) and the dose-limiting toxicities (DLTs), and to propose a safe recommended dose for further phase II investigation. Furthermore, the pharmacokinetic profile of ET-743 was investigated at all dose levels. In this article, the pharmacokinetics of ET-743, as observed in this phase I study, are described and relationships with the pharmacodynamics are evaluated.

## Patients and methods

### Patient population

Eligibility criteria included a histologically or cytologically confirmed diagnosis of a solid tumor not amenable to established forms 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 patients had acceptable bone marrow function [absolute neutrophil count (ANC) $\geqslant 2.0 \times 10^9$/l, platelets $\geqslant 100 \times 10^9$/l, hemoglobin (Hb) $\geqslant 10$ g/100 ml], adequate hepatic function [defined as serum bilirubin $< 25\,\mu$M, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) $< 3 \times$ upper normal limit or $< 2.5 \times$ upper normal limit when due to liver metastases] and adequate renal function (serum creatinine $\leqslant 120\,\mu$M and creatinine clearance $\geqslant 60$ ml/min). Other eligibility criteria were a performance status (PS) $\leqslant 2$ on the ECOG (Eastern Cooperative Oncology Group) scale, age between 18 and 75 years, and a life expectancy of $\geqslant 3$ months. The patients had to have a normal ECG and had to be recovered from any prior surgery. The study protocol was approved by the Medical Ethics Committee of the study centers and all patients gave written informed consent.

### DLTs

All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC).[23] DLTs were defined as any of the following events occurring during the first treatment cycle and attributable to ET-743: grade 3 or 4 rise in AST, ALT,

bilirubin or AP, which have not recovered to grade 0 by day 21 and still present at day 28; grade 4 neutropenia lasting longer than 5 days or complicated febrile neutropenia; any toxicity of grade 3 or above (excluding emesis, leukopenia and neutropenia).

The dose at which at least two out of three or two or more out of six patients experienced DLT was defined as the MTD. The next lower dose level below the MTD was defined as the recommended dose for phase II studies.

## Treatment plan and study design

The starting dose in this phase I, dose-escalation study was 50 $\mu$g/m$^2$, which was approximately 1/10th of the $LD_{10}$ of 200 $\mu$g/kg (600 $\mu$g/m$^2$) observed in mouse toxicology studies. The dose was administered as a 1-h i.v. infusion every 3 weeks. Dose escalation in 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 and additional patients were treated when a DLT was seen. Intrapatient dose escalation was not allowed. With the 1-h infusion schedule, nine dose levels were evaluated: 50, 100, 200, 330, 440, 585, 800, 1000 and 1100 $\mu$g/m$^2$. The MTD was reached at 1100 $\mu$g/m$^2$ and a dose of 1000 $\mu$g/m$^2$ was considered as a safe dose for phase II studies. However, in parallel phase I studies with other treatment regimes (24-h infusion and a daily infusion for 5 days),[19,22] DLTs were observed at considerable higher dose levels than with the 1-h infusion (recommended phase II dosages were 1500 and 1625 $\mu$g/m$^2$ as total doses every 3 weeks, respectively). Therefore, an amendment was added to the study protocol which endeavored to improve the toxicity profile and the dose intensity by prolonging the infusion duration from 1 to 3 h. Prolonging the infusion was not expected to enhance toxicity and therefore the starting dose for the 3-h infusion was set at 1000 $\mu$g/m$^2$. Dose escalation continued with the 3-h infusion and the following dose levels were evaluated: 1000, 1300, 1500, 1650 and 1800 $\mu$g/m$^2$.

ET-743 was supplied by Pharma Mar (Madrid, Spain) as a lyophilized powder in glass vials, containing 40 $\mu$g of ET-743 in 0.5 M phosphate buffer with mannitol (50 mg) as an excipient. The content of a vial was reconstituted with 1 ml of Water for Injection and the obtained solution was further diluted in 250 ml of a sterile 0.9% sodium chloride solution. ET-743 was administered i.v. through a peripheral or central venous access.

## Pharmacokinetics

Pharmacokinetic monitoring was performed during the first cycle of treatment. Serial blood samples (8 ml each) were collected in heparinized tubes at 15 time points: pre-infusion, at 30 and 59 min after the start of infusion, and at 5, 10 and 15 min and 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after the end of the 1-h infusion. For the 3-h infusion schedule, a similar sampling schedule was applied, except during the infusion when two samples were taken 1.5 and 3 h after the start of treatment. Plasma was obtained by immediate centrifugation of the samples (15 min, 4000 *g*). The clear supernatant was transferred into a polypropylene tube and stored at –20°C until analysis.

The quantification of ET-743 in human plasma was initially performed using a high-performance liquid chromatography assay combined with UV detection.[9] Solid-phase extraction was used as the sample pretreatment procedure and this assay provided a lower limit of quantification of 1 ng/ml. However, this was not sufficient to describe the complete plasma concentration versus time profile of ET-743 at the lower dose levels. To overcome these sensitivity problems, an assay was developed that coupled miniaturized liquid chromatography to an electrospray sample inlet and two quadruple mass analyzers (LC/ESI/MS/MS).[10] Solid-phase extraction on cyano columns was used as sample pretreatment procedure. This method was validated for ET-743 concentrations between 0.01 and 2.5 ng/ml which enabled description of the full pharmacokinetic profile. The assay was linear over this range and provided within-day and between-day precisions of less than 9.3% for all quality control samples. The average accuracy for the validated concentration range varied from 97 to 103%.

The pharmacokinetic parameters were calculated applying a non-compartmental analysis using the pharmacokinetic WinNonlin program (standard edition version 3.0, 1999). The maximum drug concentration ($C_{max}$) 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 concentration–time curve (AUC) was determined using the log-linear trapezoidal method with extrapolation to infinity using the terminal rate constant $k$ ($C_{last}/k$, where $C_{last}$ is the last measured analyte concentration). The elimination half-life ($t_{1/2}$) was calculated from the equation $0.693/k$; total plasma clearance (CL) was determined dividing the total administered dose ($\mu$g) by the AUC. The apparent volume

of distribution at steady state ($V_{ss}$) was calculated as $V_{ss}=CL \times MRT_{inf}$, where $MRT_{inf}$ is the mean residence time determined as $MRT_{inf}=(AUMC_{inf}/AUC)-(0.5 \times$ duration of infusion). The $AUMC_{inf}$ is the area under the first moment curve with extrapolation to infinity.

### Pharmacokinetic–pharmacodynamic relationships

The relationships between dose (both dose level and absolute dose) or any pharmacokinetic parameter (AUC, $C_{max}$, CL, $t_{1/2}$, $V_{ss}$) and the pharmacodynamics were explored using data of the first treatment cycle at all dose levels. Separate analyses were performed for the 1- and 3-h infusion schedules.

Hematological toxicities were evaluated using the percentage decrease (% decrease) in white blood cell count (WBC), ANC, platelets and Hb. The % decrease was calculated using the equation: (pretreatment value – value of the nadir)/pretreatment value $\times$ 100. Values for % decrease of all blood parameters were plotted against total dose ($\mu$g), dose level ($\mu g/m^2$) and the pharmacokinetic parameters, and were visually inspected for obvious relations. Possible relationships were further evaluated using Pearson's correlation coefficient.

For the investigation of relations between pharmacokinetics and hepatic toxicities, the levels of liver enzymes [ALT, AST, $\gamma$-glutamyltransferase ($\gamma$-GT), AP, lactate dehydrogenase (LDH)] and of bilirubin during the first cycle of treatment were used. Maximum levels of these enzymes were correlated to total dose, dose level and pharmacokinetic parameters, using Pearson's correlation coefficient. These maximum levels were then scored according to the NCI-CTC grading system which were also correlated to dose, dose level and pharmacokinetic parameters, using Spearman's correlation coefficient for ordinal variables. Furthermore, patients were divided in two groups, based on their NCI-CTC scores for each liver enzyme. The first group comprised patients who experienced mild liver toxicities (i.e. toxicity grade 0, 1 and 2); patients with more severe liver toxicities (grade 3 and 4) were assigned to the second group. Subsequently, pharmacokinetic parameters were tested for differences between these two groups using the independent samples *t*-test.

### Statistical analyses

Pharmacokinetic linearity between dose (expressed both as $\mu$g and $\mu g/m^2$) and AUC and CL during the first cycle was evaluated using linear regression analysis. Differences in pharmacokinetic parameters between first and second treatment cycle were evaluated using the paired *t*-test.

Baseline demographic and biochemical patient characteristics were examined as possible determinants of the pharmacokinetic parameters. Pearson's correlation coefficient and linear regression were used to investigate the relations between pharmacokinetic parameters and age, weight, height, body surface area (BSA), baseline levels of liver biochemistry tests, serum creatinine and albumin. Relations between pharmacokinetic parameters and PS were evaluated using Spearman's correlation coefficient. The influence on the pharmacokinetics of gender and of the presence of liver metastases at study entry were investigated using the independent samples *t*-test.

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

## Results

### Patients and treatment

In total, 72 patients were treated with either the 1-h schedule or with the 3-h infusion schedule (Table 1). Blood samples were taken from 71 patients during a total of 111 treatment cycles. Initially, pharmacokinetic monitoring was performed only during the first cycle of treatment. However, a phase I study, which was conducted in parallel[19,20] revealed that the transaminitis (defined as a rise in ALT and AST), which was substantial in the first treatment cycles, appeared to decrease in subsequent cycles. Therefore, it was decided to monitor second cycles as well, whenever possible, in order to investigate whether this phenomenon is caused by a change in the pharmacokinetic profile of ET-743.

Forty patients received ET-743 as a 1-h infusion at nine different dose levels between 50 and 1100 $\mu g/m^2$. At the MTD of 1100 $\mu g/m^2$, grade 4 thrombocytopenia and grade 3 fatigue were dose limiting; five patients were treated at the recommended phase II dose of 1000 $\mu g/m^2$. At the lower dose levels (50–585 $\mu g/m^2$), blood samples were analyzed using the LC-UV method, resulting in an incomplete description of the plasma concentration–time profile. These data could therefore not be used for further pharmacokinetic analyses. Samples of 15 patients (24 cycles) were analyzed using the LC/MS/

**Table 1.** Patient characteristics

| | 1-h infusion | 3-h infusion |
|---|---|---|
| No. of patients | 13 | 31 |
| Primary tumor site | | |
| colon | 2 | 7 |
| lung | 2 | 1 |
| esophagus | - | 3 |
| gastric | 2 | 1 |
| kidney | 1 | 2 |
| ovarium | – | 2 |
| uterus | 1 | 1 |
| rectum | 1 | 1 |
| unknown | 0 | 3 |
| others | 4 | 10 |
| Patient characteristics | | |
| male/ female | 6/7 | 13/18 |
| PS (0/1/2) | 6/4/3 | 5/21/5 |
| liver metastases (yes/no) | 4/9 | 17/14 |
| median age [years (range)] | 55 (42–69) | 59 (28–72) |
| weight (kg) | 73 (46–99) | 68 (48–105) |
| height (cm) | 167 (152–178) | 168 (154–187) |
| BSA ($m^2$) | 1.8 (1.4–2.2) | 1.8 (1.5–2.2) |
| Baseline biochemistry | | |
| ALT (U/l) | 27 (7–46) | 18 (3–74) |
| AST (U/l) | 21 (10–73) | 22 (6–78) |
| AP (U/l) | 168 (77–292) | 122 (54–539) |
| bilirubin ($\mu$mol/l) | 9 (5–16) | 8 (4–18) |
| $\gamma$-GT (U/l) | 45 (14–191) | 51 (8–594) |
| LDH (U/l) | 293 (154–1777) | 393 (108–4797) |
| serum creatinine ($\mu$mol/l) | 78 (56–108) | 69 (41–109) |
| sodium (mmol/l) | 139 (134–143) | 138 (134–143) |
| potassium (mmol/l) | 4.1 (3.9–4.9) | 4.4 (3.8–5.0) |
| albumin (g/l) | 38 (30–44) | 37 (26–47) |

Values are presented as medians (range); for abbreviations: see text

MS method at the higher dose levels (800, 1000 and 1100 $\mu g/m^2$) and complete pharmacokinetic profiles were obtained. In nine of these 15 patients, samples were taken during both the first and second cycles, and in four patients only the first cycle was pharmacokinetically monitored; in the remaining two patients only the samples from the second cycles were analyzed using the LC/MS/MS method. Of these two patients, samples from one were only taken up to 7 h after the start of infusion; this patient was not included in further analyses, as a complete pharmacokinetic profile was not available. The other patient was included only in the pharmacokinetic analyses, as covariate and pharmacodynamic analyses were performed only with data in the first treatment cycle. In summary, pharmacokinetic data of 14 patients in 23 cycles were available for the 1-h infusion schedule (Table 2).

Dose escalation for the 3-h infusion schedule was at five dose levels between 1000 $\mu g/m^2$ and the MTD of 1800 $\mu g/m^2$ (Table 3) at which the DLTs were pancytopenia and fatigue. The recommended phase II dose, derived from the present data, was 1650 $\mu g/m^2$ given over 3 h. An extended cohort of 12 patients was treated at the recommended dose in order to further set down the pharmacokinetic and toxicological profile, before commencing extensive phase II investigations. All 31 patients treated over 3 h were pharmacokinetically sampled in a total of 54 cycles. Bio-analysis was performed using the LC/MS/MS method. In two patients, only few samples were taken during the second cycle and those curves were not included in the pharmacokinetic analyses. One patient had a dose reduction during cycle 2 so data from the second cycle were not used for further analyses.

Patient characteristics of all patients available for pharmacokinetic analyses are listed in Table 1.

## Pharmacokinetics

Pharmacokinetic parameters of ET-743 were calculated at the three highest dose levels for the 1-h

**Table 2.** Pharmacokinetic parameters of ET-743 administered as a 1-h infusion (values for the first and the second cycle are presented as mean±SD)

| Dose level ($\mu$g/m$^2$) | Cycle | *N* | AUC (h · ng/ml) | CL (l/h) | $C_{max}$ (ng/ml) | $t_{1/2}$ (h) | $V_{ss}$ (L) |
|---|---|---|---|---|---|---|---|
| 800 | 1 | 4 | 23±7.5 | 58±25 | 13±6.9 | 50 (±63) | 2000 (±2500) |
| 1000 | 1 | 5 | 36±6.4 | 32±6.1 | 17±5.2 | 33 (±14) | 910 (±720) |
| 1100 | 1 | 4 | 52±16 | 25±9.0 | 18±4.5 | 36 (±13) | 980 (±800) |
| 800 | 2 | 2 | 22±11 | 62±31 | 10±3.0 | 65 (±71) | 4200 (±3400) |
| 1000 | 2 | 4 | 26±18 | 60±26 | 11±1.2 | 28 (±26) | 1700 (±330) |
| 1100 | 2 | 4 | 56±34 | 52±67 | 25±15 | 24 (±8) | 2300 (±3600) |

**Table 3.** Pharmacokinetic parameters of ET-743 administered as a 3-h infusion (values for the first and the second cycle are presented as mean±SD)

| Dose level ($\mu$g/m$^2$) | Cycle | *N* | AUC (h · ng/ml) | CL (l/h) | $C_{max}$ (ng/ml) | $t_{1/2}$ (h) | $V_{ss}$ (L) |
|---|---|---|---|---|---|---|---|
| 1000 | 1 | 3 | 31±16 | 66±29 | 5.1±1.2 | 46 (±31) | 2200 (±1300) |
| 1300 | 1 | 6 | 25±8 | 100±26 | 5.4±1.7 | 28 (±19) | 2000 (±1600) |
| 1500 | 1 | 6 | 59±29 | 51±24 | 10±3.7 | 43 (±42) | 1500 (±1600) |
| 1650 | 1 | 12 | 38±10 | 87±30 | 8.6±2.5 | 26 (±7) | 1400 (±490) |
| 1800 | 1 | 4 | 71±38 | 55±30 | 12±6.8 | 31 (±14) | 1500 (±1200) |
| 1000 | 2 | 3 | 31±10 | 61±22 | 6.4±1.5 | 36 (±17) | 1600 (±1000) |
| 1300 | 2 | 4 | 31±11 | 84±27 | 6.4±1.8 | 29 (±8) | 1600 (±170) |
| 1500 | 2 | 3 | 29±4 | 81±11 | 7.4±1.5 | 16 (±0.81) | 760 (±160) |
| 1650 | 2 | 9 | 55±22 | 63±33 | 10±3.5 | 36 (±30) | 1600 (±1200) |

infusion (800, 1000 and 1100 $\mu$g/m$^2$) and at all dose levels for the 3-h infusion, during both the first and second cycles of treatment where possible. Mean values (SD) for both treatment schedules are presented in Tables 2 and 3. In Figure 2, plasma concentration versus time curves at the three highest dose levels for the 1-h infusion are shown. Plasma concentration versus time profiles for the 3-h infusion at 3 different dose levels (1000, 1650 and 1800 $\mu$g/m$^2$) are presented in Figure 3.

Values for CL, as calculated for the first cycle of the 1-h infusion schedule, decreased with dose ($r$=–0.76, $p$=0.003), indicating non-linear pharmacokinetics during the first cycle using this schedule. When both first and second cycle data were taken into account, CL decreased with dose, although this decrease was not statistically significant ($r$=–0.17, $p$=0.437). At the 3-h infusion, CL was constant and not significantly correlated to dose ($r$=–0.72, $p$=0.702), and a linear increase of AUC with dose was observed ($r$=0.18, $p$=0.018). Figure 4 illustrates the relation between AUC, as calculated for the 3-h schedule, and dose. As can be derived from Tables 2 and 3 and Figure 4, interpatient variability was considerable in this study (e.g. 14–69% for AUC).

The 1000 $\mu$g/m$^2$ dose was given both as a 1-h ($n$=5 patients) and as a 3-h ($n$=3 patients) infusion. The mean value for $C_{max}$ at this dose level reached with the 1-h infusion (17 ng/ml) was approximately 3-fold higher than with the 3-h infusion (5.1 ng/ml). However, the mean CL for the 1-h infusion was reduced in comparison with the 3-h schedule (32 and 66 l/h, respectively).

The influence of prior exposure to ET-743 on the pharmacokinetics of subsequent cycles was evaluated for both treatment schedules by comparing pharmacokinetic parameters (CL, $V_{ss}$, AUC, $t_{1/2}$, $C_{max}$) between the first and second cycle at each dose level, using the paired $t$-test. For the 1-h infusion, paired values for pharmacokinetic parameters were available at three dose levels: 800 ($n$=2), 1000 ($n$=3) and 1100 $\mu$g/m$^2$ ($n$=3). With this schedule, pharmacokinetic parameters did not differ significantly between the first and the second cycles of treatment. For the 3-h infusion, pharmacokinetic parameters of the first and second cycles could be compared at four dose levels: 1000 ($n$=3), 1300 ($n$=4), 1500 ($n$=3) and 1650 ($n$=9) $\mu$g/m$^2$. At the dose of 1800 $\mu$g/m$^2$, only a single patient was pharmacokinetically monitored in both cycles and therefore excluded for analysis of any effects of prior exposure. At a dose of 1000 $\mu$g/m$^2$, higher values for $C_{max}$ were observed in the second treatment cycle (Table 3), which was statistically significant ($p$=0.019). Values for AUC at the 1650 $\mu$g/m$^2$ level were significantly higher ($p$=0.005) in the second cycle as compared to the first treatment cycle (55 and 38 h ng/ml, respectively). The corresponding values for CL at this dose level were significantly ($p$=0.005) lower in the second

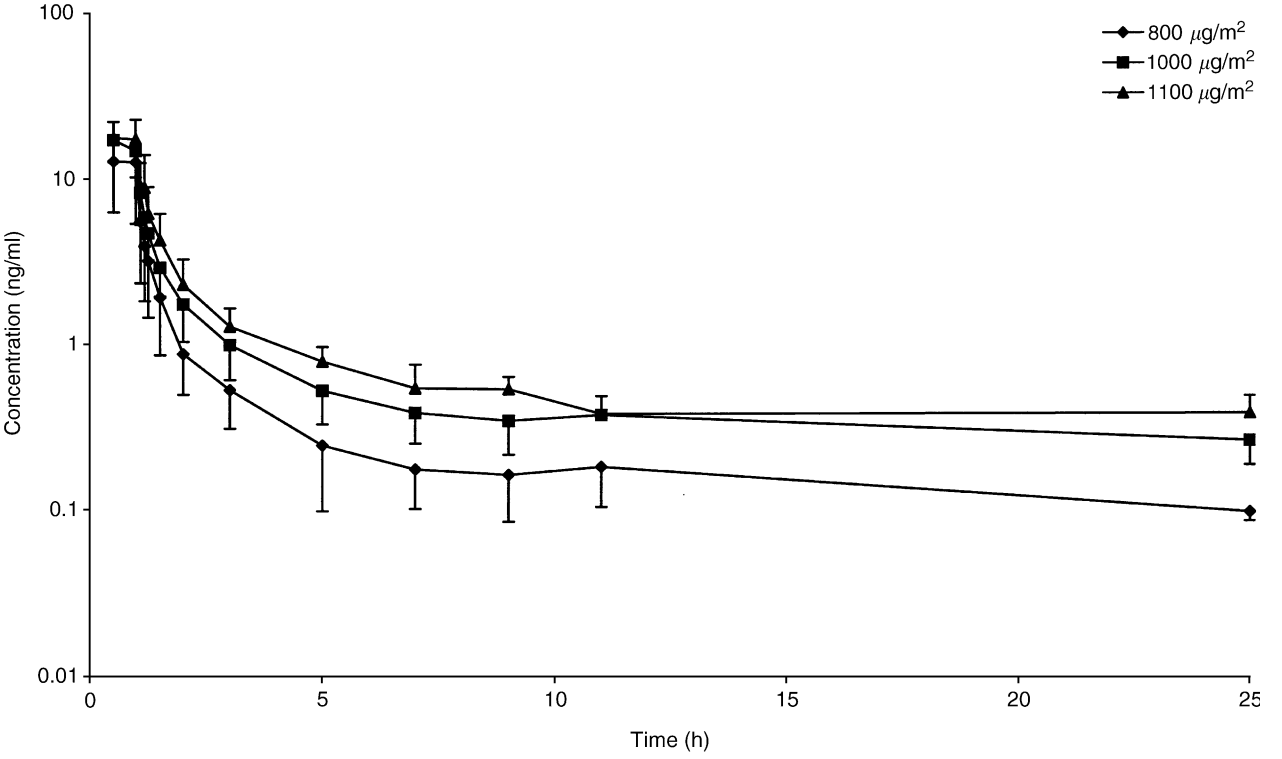


**Figure 2.** Plasma concentration versus time profile of patients treated with the 1-h infusion schedule of ET-743 during the first cycle of treatment. Mean values±SD are depicted for three dose levels (800,1000 and 1100 $\mu g/m^2$).

cycle as compared to the first cycle (63 and 87 l/h, respectively).

Patient characteristics were investigated as possible determinants of interpatient pharmacokinetic variability. With the 1-h infusion ($n$=13), CL was significantly correlated to BSA ($r$=0.68, $p$=0.011). Values for $V_{ss}$ were significantly correlated to PS ($r$=0.59, $p$=0.035) and baseline levels of AP ($r$=−0.56, $p$=0.046). For the 3-h treatment schedule ($n$=31), significant correlations were detected between $V_{ss}$ and PS ($r$=−0.48, $p$=0.007).

The presence of liver metastases did not alter the pharmacokinetic profile of ET-743; there were no significant differences in any pharmacokinetic parameter between patients with or without liver metastases for either the 1- or 3-h infusion schedule. Furthermore, the pharmacokinetic profile of ET-743 did not differ significantly between male and female patients in the present study.

## Pharmacokinetic–pharmacodynamic relationships

Pharmacokinetic parameters (AUC, $C_{max}$, CL, $t_{1/2}$, $V_{ss}$) of all patients during the first cycle of treatment and the corresponding dosages [both dose ($\mu g/m^2$) and total dose ($\mu g$)] were investigated for their relations to % decrease in ANC, WBC, platelets and Hb. The 1-h infusion schedule in this study showed significant correlations between the % decrease in WBC and AUC, $C_{max}$ and dose level ($\mu g/m^2$); the % decrease in ANC was also significantly correlated with $C_{max}$. There was no evidence of a relationship between pharmacokinetic parameters and other hematological toxicities. For the 3-h infusion, the percentage decrease in ANC, WBC and platelets appeared to be significantly correlated to AUC, $C_{max}$ and dose ($\mu g$). Furthermore, the percentage decrease in ANC significantly increased with dose level ($\mu g/m^2$). In Figure 5(A–C), the percentage decreases in WBC, ANC and platelets are plotted versus AUC. With the 3-h infusion, the decrease in Hb, which was only minor, was not significantly correlated to dose, dose level or any pharmacokinetic parameter.

In general, an elevation in liver biochemistry tests (AST, ALT, AP, bilirubin, $\gamma$-GT, LDH) up to grade 4, was observed with increasing dose, dose level and values for AUC and $C_{max}$. This effect was found with both treatment schedules, using either absolute values or NCI-CTC scores. For the 1-h infusion, no

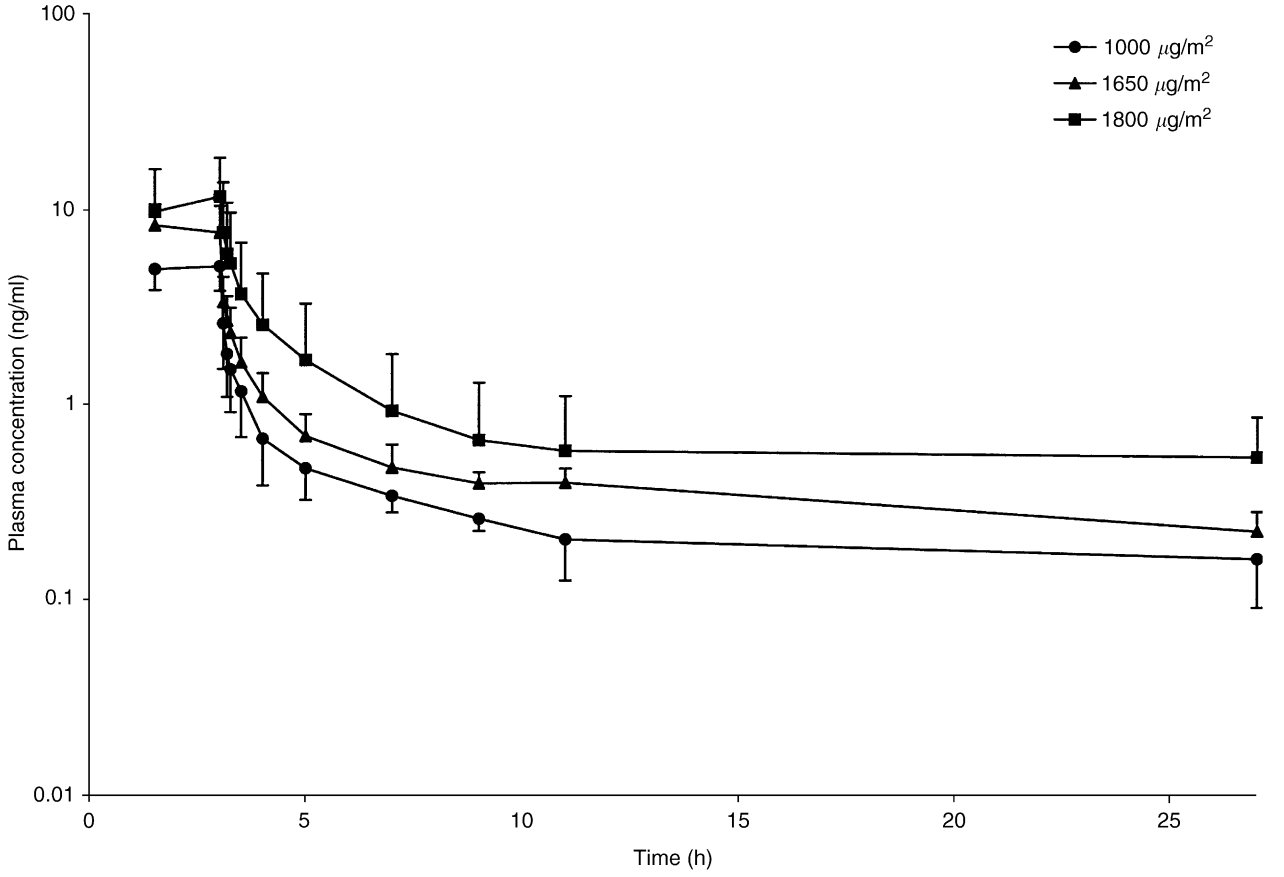


**Figure 3.** Plasma concentration versus time profile of patients treated with the 3-h infusion schedule of ET-743 during the first cycle of treatment. Mean values±SD are depicted for three dose levels (1000, 1650 and 1800 $\mu g/m^2$).

differences in pharmacokinetic parameters were observed between patient groups with mild or severe toxicities. However, with the 3-h infusion, patients experiencing a grade 3 or 4 elevation in AP and AST during the first cycle showed significantly higher $C_{max}$ values than patients with grade 2 or greater rise in AP and AST ($p=0.02$ for AP and $p<0.04$ for AST) (Figure 6).

## Discussion

In this phase I study, the pharmacokinetic properties of ET-743 were evaluated when administered as a 1- and 3-h infusion. A total of 72 patients was treated at different dose levels and over multiple cycles; data of 45 patients could be used for full pharmacokinetic analyses. With ET-743 administered as a 1-h infusion, the MTD was 1100 $\mu g/m^2$ and the potential recommended phase II dose was 1000 $\mu g/m^2$. DLTs were severe thrombocytopenia and fatigue. As this MTD was substantially lower than in other phase I studies which were conducted in parallel, dose escalation continued using a 3-h infusion. With this treatment schedule, the MTD was higher at 1800 $\mu g/m^2$, with the DLTs pancytopenia and fatigue. Hepatic toxicity was observed with both schedules, but it was not dose limiting, appeared to be transient and was not cumulative. This toxicological profile is consistent with the findings in preclinical experiments.[1,8] From the results of the present study a recommended dose of 1650 $\mu g/m^2$ given over 3 h was chosen for phase II studies and an extended cohort of patients was entered at this dose level, to further characterize the safety profile of the drug. Promising antitumor activity was seen with this treatment schedule including responses in soft-tissue sarcomas.[24]

Pharmacokinetic properties of ET-743 have been described for different treatment schedules, i.e. 24- and 72-h infusions.[19,21] ET-743 showed linear pharmacokinetics over the dose range tested in the 24-h infusion (i.e. 50–1800 $\mu g/m^2$). However, a prolonged 72-h administration of ET-743 suggested a departure

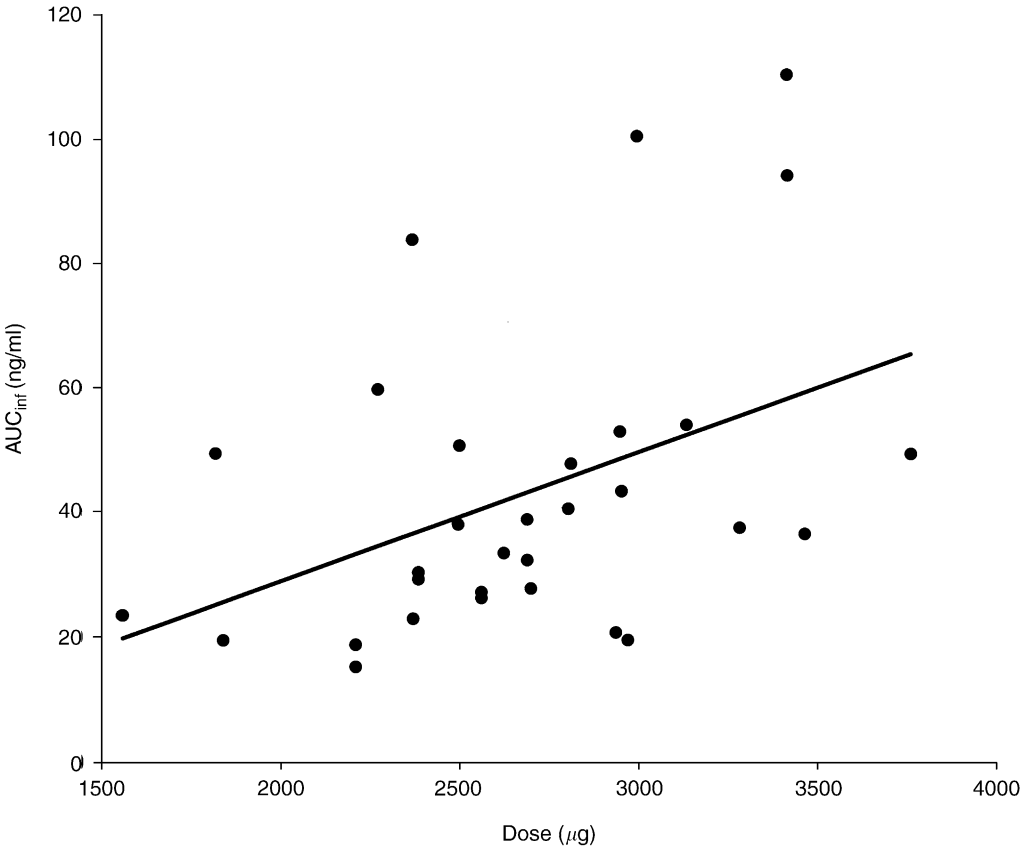


**Figure 4.** AUC versus dose during the first cycle of the 3-h infusion.

from linear pharmacokinetic behavior at doses exceeding 1050 $\mu$g/m$^2$, as a disproportional increase in AUC and $C_{max}$ was observed with the MTD of 1200 $\mu$g/m$^2$. Pharmacokinetic analyses in the present study also indicate that the pharmacokinetics of ET-743 may be non-linear as a decrease in CL was seen with increasing doses in the 1-h infusion schedule. By contrast, with the 3-h infusion schedule CL was constant over the dose range tested, and the increases in AUC and $C_{max}$ were linear. These results suggest that the pharmacokinetic properties of ET-743 may be dependent on the treatment schedule and on the dose. As in the present study, the 1000 $\mu$g/m$^2$ dose of ET-743 was given both as a 1- and 3-h infusion, this could be further investigated by comparing the calculated pharmacokinetic, parameters of both treatment schedules. The mean $C_{max}$ was approximately 3-fold higher with the 1-h infusion. However, the mean value for CL with this schedule was lower than for the 3-h infusion (32 and 66 l/h, respectively), implying that elimination processes may be saturable when ET-743 is administered as a 1-h infusion. This is supported by the fact that, although the MTD for the 1-h schedule was substantially lower than for the 24-h infusion (1100 and 1800 $\mu$g/m$^2$, respectively), the mean AUC achieved was comparable (52 and 50 h · ng/ml).[19] As a possible explanation for the non-linear pharmacokinetics observed with the 72-h infusion, it was suggested that liver damage occurs during the infusion which may have an impact upon the elimination of the remaining fraction of the drug.[21] However, the non-linearity with the present 1-h infusion is more likely to be caused by another underlying mechanism, e.g. a saturation of the metabolic enzymes due to the very high plasma concentrations were reached with this infusion schedule. In summary, indications for non-linear characteristics in the pharmacokinetics of ET-743 were obtained, but further, e.g. population pharmacokinetic, analyses combining data of different treatment schedules could contribute to a more profound description of the pharmacokinetic, profile.

The possible schedule dependency of ET-743 pharmacokinetics could explain why the MTD with the 1-h infusion was reached at a lower dose level than in other phase I studies that evaluated prolonged infusion schedules,[19,21] although it is more

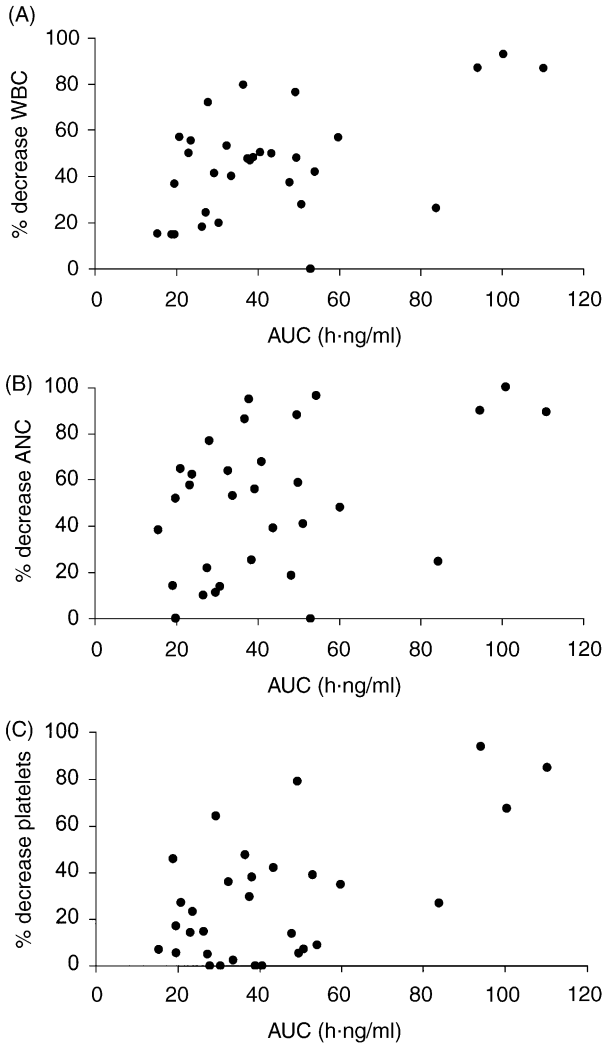


**Figure 5.** Percentage decrease in WBC, ANC and platelets during the first cycle with the 3-h infusion versus AUC.

likely that the difference in MTD is caused by the higher plasma concentrations that were seen with the 1-h infusion compared to prolonged infusions. The values for $C_{max}$ that were reached at the MTD for the 1-h schedule (18 ng/ml) were approximately 10-fold higher than at the MTD for the 24-h schedule (1.9 ng/ml).[19] With the 72-h infusion schedule, the $C_{max}$ at the MTD was 0.66 (ng/ml) and comparable or higher values for the mean AUC were seen (72 h · ng/ml) than at the MTD with the 3- and 24-h infusion (71 and 50 h · ng/ml, respectively). Hepatic toxicity and not myelosuppression was considered a DLT in a 72-h schedule, and it therefore seems likely that hematological toxicity may be related to $C_{max}$.[21]

At all dose levels and with both treatment schedules, high values for $V_{ss}$ were observed. Apparently, ET-743 is extensively distributed in the body, followed by a slow redistribution and elimination which is reflected by the long terminal half-life of the drug (26 h at the recommended dose of 1650 $\mu$g/m$^2$).

Initially, ET-743 pharmacokinetics were studied only during the first cycle of treatment for each patient. However, other trials in the phase I program suggested that increases in transaminase levels, which reached grade 3 in the majority of the patients during the first cycle, diminished in consecutive cycles.[20] To investigate whether this rather unexpected finding could be explained by, or lead to, a change in the pharmacokinetics of ET-743, samples were subsequently collected during the second cycle in the present study. For the 1-h infusion schedule no significant differences were detected between pharmacokinetic parameters of the first and second cycle. With the 3-h infusion it appeared that at a dose of 1000 $\mu$g/m$^2$, values for $C_{max}$ were significantly lower during the first cycle. Furthermore, at the 1650 $\mu$g/m$^2$ level, CL is decreased during the second cycle and the corresponding values of AUC are increased. However, at the other dose levels in this study, pharmacokinetic parameters were essentially the same for both cycles. In summary, it cannot be concluded that the pharmacokinetic profile is altered by previous exposure to ET-743 and these results do not explain the diminished hepatic toxicity in later treatment cycles. Both the 24-h infusion study and the 72-h infusion study with ET-743 also revealed no clear difference in pharmacokinetic profile between treatment cycles.[19,21]

The interpatient variability in ET-743 pharmacokinetics in this study was considerable (e.g. 14–69% for AUC). The possible influence of patient characteristics and renal function and liver biochemistry tests at study entry on this variability was explored. Values for CL, as calculated with the 1-h infusion, were significantly correlated to BSA. However, this relation could not be detected in the 3-h infusion schedule and therefore it remains unclear whether individual adaptation of the dose based on BSA would be beneficial. The presence of liver metastases at study entry did not alter the CL of ET-743. No other clinically relevant correlations could be revealed with biochemistry, renal and liver function at study entry, for both treatment schedules. However, the study entry criteria excluded patients with substantially impaired renal function or deranged liver biochemistry tests so an effect of ET-743 cannot be entirely ruled out.

The present study revealed significant linear correlations between % decrease in ANC, platelets and WBC with AUC, $C_{max}$ and dose. The effect of ET-

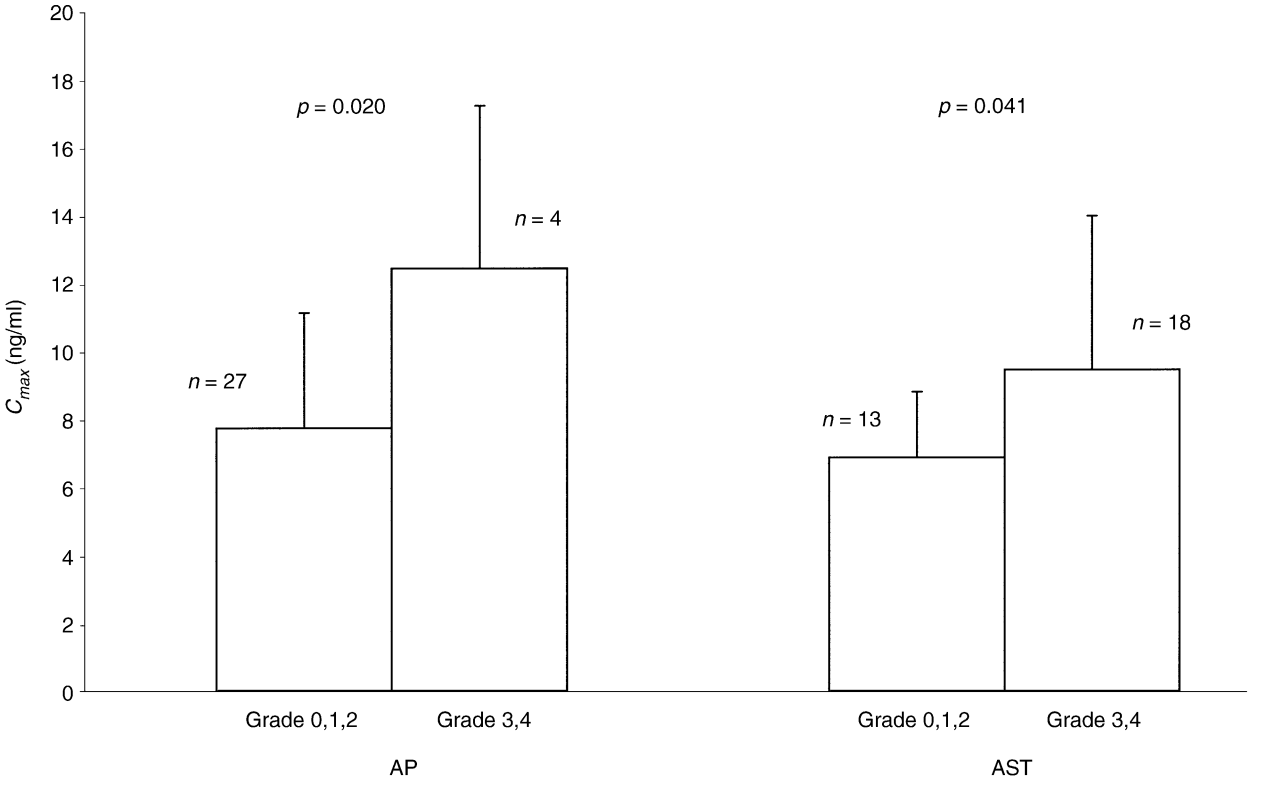


**Figure 6.** Mean values for the $C_{max}$ (±SD) observed in patients experiencing grade 2 or lower AP/AST elevation or grade 3 or higher ALT/AST elevation. Values for both ALT and AST are presented.

743 on Hb was modest and therefore no relations were noted with this pharmacodynamic parameter. Although correlations between kinetic parameters and fall in ANC, WBC and platelets were clearly noted, the variability was considerable (Figure 5). Therefore, it cannot be stated now which parameter (AUC, $C_{max}$ or dose) best predicts hematological toxicity. The relationships described here were estimated using linear correlations (Pearson's correlation coefficient). This may not be an optimal model as it can be assumed that the % decrease in cells ranges from 0 to 100%. Other models (e.g. logarithmic or a sigmoidal maximum effect model) could be applied,[25,26] although the shape of the relationship cannot be clearly determined in the current study, due to the large variability. Interestingly, for the 24-h infusion schedule, a clear relationship was noted between the % decrease in ANC and the AUC,[19] which was adequately described by a sigmoidal Emax model.

Hepatic toxicity increased with dose and values for AUC and $C_{max}$, although it was not dose limiting as all elevations had recovered fully prior to the next treatment cycle. Preclinical studies also revealed reversible hepatic toxicity at doses below the $LD_{10}$, but the mechanism remains unclear.[1] Patients receiving the 3-h treatment schedule who experienced a grade 3 or 4 increase in AST and AP showed significantly higher values for $C_{max}$ than patients with milder toxicity (grade 0, 1 or 2). Combined analyses of hepatic toxicity and pharmacokinetics for the 24-h infusion study revealed that patients with grade 3 or 4 AST or ALT elevation experienced significantly higher values of AUC.[19] With the 72-h infusion, grade 4 transaminitis was considered a DLT and the rise in AST levels was significantly correlated to AUC.[21] By contrast, in the present study, marked increases in liver enzymes were clearly related to $C_{max}$ (9 ng/ml at the recommended dose of 1650 $\mu$g/m$^2$), which was substantially higher than with the 24- and 72-h schedules (1.8 and 0.32 ng/ml at the recommended dose in both studies, respectively).

Data on the metabolism of ET-743 are scarce and so far its 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,[27] although metabolic products could not be identified. These studies suggest that in both rats and humans the cytochrome P450 enzymes from the 3A subfamily are mainly responsible for the observed metabolic activity. ET-743 metabolic clearance after incubation with male rat liver microsomes appeared to be

substantially higher than with female microsomal preparations.[27] This is probably due to the male predominance of the responsible enzyme (CYP3A2) in the rat. These differences in metabolism might contribute to the higher sensitivity of female rats for ET-743 that was seen during *in vivo* toxicity studies.[1] It is unlikely that this difference in rate of metabolism between male and female rats would be observed in humans where cytochrome P450 enzymes do not exhibit such gender differences.[27] Indeed, the present clinical study confirmed these findings as no significant difference was found for CL or $t_{1/2}$ between male and female patients, at both treatment schedules. Recently, the metabolism of ET-743 was investigated both *in vitro* (incubations with plasma, urine, human microsomes and 5′-diphospho-glucuronyltransferase) and *in vivo* (in bile, plasma and urine of treated patients).[28] Those experiments have shown a major breakdown of ET-743; however, the main reaction products were formed non-enzymatically. Traces of deacetylated ET-743 were found in plasma. Glucuronidated ET-743 could be synthesized *in vitro* but were not be detected in bile, urine or plasma of treated patients. This is in agreement with data of a patient with Gilbert's syndrome treated with ET-743 showing no pharmacokinetic nor pharmacodynamic differences with other treated patients.[28]

The present phase I trial indicates that ET-743, administered as a 3-h infusion every 3 weeks, is well tolerated, with the DLTs being pancytopenia and fatigue. With this schedule, we observed linear pharmacokinetics over the dose range tested, with considerable interpatient variability. Hepatic and hematological toxicity were related to exposure to ET-743. Preclinical studies revealed potent activity of ET-743 at nanomolar concentrations.[5–7] At the recommended dose with the 3-h schedule plasma concentrations of the same order of magnitude were reached in patients. Although possible differences in drug sensitivity and differences in the schedule of administration may hinder extrapolation to patients, these are promising results and antitumor activity was seen in the current study.[24] Phase II studies have already commenced with this schedule in different tumor types, including melanoma, breast, ovarian and colon cancer.

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