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Phase I and pharmacokinetic study of Yondelis™ (Ecteinascidin-743; ET-743) administered as an infusion over 1 h or 3 h every 21 days in patients with solid tumours

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Abstract

Yondelis™ (ET-743) is a novel anticancer agent isolated from the marine ascidian *Ecteinascidia turbinata*. ET-743 possesses potent antitumour activity and a novel mechanism of action at the level of gene transcription. We conducted two sequential phase I dose escalation and pharmacokinetic studies of ET-743 given as a 1- or a 3-h intravenous (i.v.) infusion. Seventy-two adults with metastatic or advanced solid tumours received ET-743 in escalating doses between 50 and 1100 μg/m^2, initially as a 1-h infusion, and later at doses between 1000 and 1800 μg/m^2 as a 3-h infusion every 3 weeks. The maximum tolerated dose (MTD) of ET-743 was 1100 μg/m^2 for the 1-h infusion schedule and 1800 μg/m^2 when given as a 3-h infusion. Dose-limiting toxicities (DLTs) were fatigue, neutropenia and thrombocytopenia. Transient non-cumulatives grade 3–4 increase in transaminases (not considered DLT) and grades 3–4 nausea and vomiting were frequently observed. Other toxicities (maximum grade 3) included anaemia, increased lactate dehydrogenase (LDH), bilirubin and alkaline phosphatase serum levels, and phlebitis; there were no toxic deaths. One pCR (melanoma), CR (uterine leiomyosarcoma), one PR (colon stromal sarcoma) and a MR (37% tumour shrinkage, gastric stromal sarcoma) were observed. A further 9 patients with colorectal, mesothelioma, bile duct carcinoma and bladder cancer had SD which lasted for six or more treatment cycles. ET-743 pharmacokinetics were linear with the 3-h infusion schedule. The haematological and hepatic toxicities of ET-743 were dose-dependent and not cumulative. Based on the current trial, the recommended dose of ET-743 for phase II studies is 1650 μg/m^2 given as a 3-h infusion.

Keywords: Ecteinascidin-743; ET-743; Phase I study; Human pharmacokinetics; Solid tumours

1. Introduction

Yondelis™ (Ecteinascidin-743 or ET-743), a novel antitumour agent isolated from the marine ascidian *Ecteinascidia turbinata,* has considerable antitumour activity in murine and human tumours *in vitro* [1,2]. Potent antitumour activity has been demonstrated in a broad range of *in vivo* tumour models, including human tumour xenografts in nude mice [3–5].

ET-743 acts by a novel, complex mechanism predominantly at the level of gene transcription. ET-743 binds to guanine–cytosine rich sequences in the minor groove of DNA and alkylates guanine residues at the

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N2 position [6]. Cell cycle studies have demonstrated that ET-743 decreases the rate of progression of tumour cells through S-phase and causes prolonged p53-independent blockade in G_2/M, giving rise to a strong apoptotic response [7]. Cells in G_1 are more sensitive to the cytotoxic effects of ET-743 than cells in S-phase or G_2/M [8]. These effects appear to be mediated by multiple mechanisms. ET-743 inhibits activation of the transcription of certain genes relevant to the process of the mdr-1 resistant pathway and nucleotide excision repair mechanism [8–11].

Toxicological evaluations of ET-743 as a single or a fractionated dose by the intravenous (i.v.) route in mice, rats, and dogs have consistently shown the potential of ET-743 to induce reversible haematological and hepatic toxicity. Hepatotoxicity was evident from transient increases in serum levels of liver enzymes, bilirubin and bile acids, and from histopathological changes in the liver. Further toxicity included lesions at the site of injection, spleen and thymus lesions, bile duct hyperplasia and decreased testicular and ovarian weights. Studies in dogs showed vomiting and diarrhoea following the administration of ET-743. A study in cynomolgus monkeys confirmed the potential of single doses of ET-743 to induce hepatic and haematological toxicity, emesis and diarrhoea. However, fractionated dosing induced only minor toxicity in dogs [12].

An *in vitro* bone marrow assay using human, murine and canine progenitor cells, showed equal sensitivity of erythropoietic and myeloid cells to ET-743. Prolonged or repeated exposure to the drug proved more toxic to haematopoietic progenitors than a single 1-h exposure [13]. The therapeutic index of ET-743 was more favourable with a prolonged exposure.

A clinical development programme of ET-743 in cancer patients was started with phase I studies investigating 1-, 3-, 24- and 72-h i.v. infusion schedules and a daily $\times 5$ (d$\times 5$) schedule. Results of the 3-, 24- and 72-h and d$\times 5$ studies have been reported recently [14–19]. Here, we report the phase I study investigating the 1-h schedule repeated every 3 weeks. The study was amended to continue with a 3-h infusion duration when the maximum tolerated dose (MTD) for the 1-h infusion had been reached.

2. Patients and methods

ET-743 was first administered as a single 1-h i.v. infusion every 21 days in a conventional open-label, non-randomised phase I dose escalation study. The primary aim was to determine the MTD of this dose schedule, defined as that producing dose-limiting toxicity (DLT) in $\geqslant 2/6$ patients in the first cycle. DLT was defined as:

- Grade 4 neutropenia that lasted more than 5 days, was complicated or associated with fever.
- Grade 3 or 4 elevation of transaminases, bilirubin or alkaline phosphatase, which had not returned to baseline at day 28.
- Other toxicity of grade 3 or more, excluding emesis.

Further aims of the study were to propose a safe dose for phase II evaluation, to describe the qualitative and quantitative toxic effects, to study the pharmacokinetics in man at different dose levels and to document the possible antitumour activity of ET-743 given as a 1-h i.v. infusion.

After the MTD for the 1-h infusion had been reached, the study was extended by protocol amendment, enabling further patients to be treated with a 3-h infusion of ET-743. The aim of this extension was to determine whether a higher dose would be tolerated when the infusion duration was prolonged to 3 h. The decision to extend the study was taken based on the likelihood that dose-limiting toxicities were C_{max}-related and because the long terminal half life of the drug made use of relatively short infusion times possible. It was, therefore, decided to evaluate 3 h as this was the longest infusion time compatible with an out-patient administration. In addition, in parallel, another phase I study investigating ET-743 as a 24-h infusion, had yielded a higher MTD and recommended dose for phase II trials than the 1-h infusion in this study [14].

Patients aged 18 years or above with a histologically- or cytologically-confirmed diagnosis of solid tumour were eligible, if they were not amenable to established forms of treatment. Further inclusion criteria were: performance status (PS) $\leqslant 2$ Eastern Cooperative Oncology Group (ECOG) scale, life expectancy $\geqslant 3$ months, neutrophil count $\geqslant 2.0\times 10^9$/l, platelet count $\geqslant 100\times 10^9$/l, haemoglobin $\geqslant 10$ g/100 ml, normal hepatic function except for patients with liver metastases (who were eligible if aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $<2.5\times$ the upper limit of normal (UNL), normal renal function, normal electrocardiogram (ECG) and negative serology for hepatitis. Exclusion criteria were those routinely employed in phase I studies of cytotoxic agents with the potential to induce bone marrow suppression. Patients were required to have given informed consent before being entered into the study.

ET-743 was provided as a lyophilised powder in glass vials (PharmaMar, Tres Cantos, Madrid, Spain) containing 40 or 250 mcg of ET-743. The drug product was reconstituted with 1 or 5 ml of sterile water, respectively, and diluted further with saline for injection to a volume of 250–1000 ml. The resulting solution was infused over 1 or 3 h through a peripheral or central i.v. line. Drug administration was repeated every 3 weeks

provided the patient had recovered to grade 1 from any toxicity of the preceding course.

A dose of 50 μg/m^2 (equivalent to one-tenth of the MTD in mice and rats and less than one-third of the non-toxic level in dogs) was considered a safe starting dose on the basis of acute toxicity studies in animals. Dose escalation was based on a modified Fibonacci's scheme according to the safety profile observed at the last dose level with data for at least 3 patients and four cycles of treatment available. Within-patient dose escalation was not permitted. Dose escalation continued until significant toxicity was observed in which case further patients (to a total of at least 6 patients) were entered at the same dose level. Additional patients were to be treated at the proposed phase II dose to confirm the toxicity profile of the recommended dose.

Prestudy and repeated assessments during treatment included medical history, physical examination, neurological evaluation, PS, full blood count, blood biochemistry profile, urinalysis, ECG, vital signs, tumour size measurements by radiological imaging (every 6 weeks) and chest X-ray (or other radiological imaging) to determine the extent of disease. In patients experiencing grade 3 or more liver toxicity, blood biochemistry was to be performed twice weekly until values had normalised. Toxicity assessments were continued until 30 days after the last treatment or until resolution of all toxicities occurring during treatment with ET-743. Toxicity was graded according to the National Cancer Institute of Canada (NCIC) Expanded Common Toxicity Criteria rating scale. Tumour responses were rated as complete responses (CR), partial response (PR), no change (NC) or progressive disease (PD) according to World Health Organization (WHO) criteria [23].

This was a collaborative study by two clinical centres in the UK and one in The Netherlands conducted according to the standard operating procedures of NDDO Oncology (Amsterdam, The Netherlands) for the New Drug Development Group of the EORTC. The study protocol was approved by the Ethics Committees of the participating institutions. NDDO Oncology served as the central coordinating and administrative office and was also in charge of monitoring of adherence to the protocol and the provisions of Good Clinical Practice.

Details of the pharmacokinetic part of this study have been described elsewhere [20].

Serial blood samples (8 ml each) were taken during the first cycle of treatment in each patient at various time intervals before, during and after the drug infusion. Blood samples were collected in heparinised plastic tubes and centrifuged at 4000*g* for 15 min immediately following collection. Plasma was transferred to polypropylene tubes, which were labelled and stored at −20 °C until analysis. At the lower dose levels of the 1-h infusion (<800 μg/m^2), quantitation of ET-743 in plasma samples was performed using a high performance liquid chromatography (HPLC) assay with ultraviolet (UV) detection and a lower limit of detection 1 ng/ml [21]. Since incomplete plasma curves were obtained using this method, the more sensitive miniaturised liquid chromatography assay with MS/MS detection and a lower limit of quantitation of 10 pg/ml [22] was used to analyse all subsequent plasma samples.

3. Results

3.1. Patients' characteristics and treatments administered

72 patients were enrolled in the two parts of the study between June 1996 and June 2000. The patient population is summarised in Table 1. Tumours of the gastrointestinal tract were the most common and almost all patients had received prior chemotherapy (85%) or radiotherapy (29%).

Cohorts of 3–6 patients were entered at doses escalating from 50 to 1100 μg/m^2 using the 1-h infusion (Table 2). Since 1100 μg/m^2 appeared to be the MTD (see below), 8 further patients were entered at a dose of 1000 μg/m^2. A subsequent cohort of 3 patients was entered at this dose administered as a 3-h infusion and further cohorts of 4–6 patients at doses of 1300–1800 μg/m^2 as a 3-h infusion. Since 1800 μg/m^2 was the MTD for the 3-h infusion (see below), a further 13 patients were entered at the 1650 μg/m^2 dose level, this being the presumed recommended dose for phase II studies using this schedule.

Patients received a total of 227 cycles of treatment, 124 cycles with the 1-h infusion and 103 cycles with the 3-h infusion (Table 2). Patients received a median of two treatment cycles with a range of 1–10 cycles and 1–17 cycles for the 1- and 3-h infusion schedules, respectively. Overall, 19% of courses were delayed, almost always by 2–14 days. Dose delays occurred in 14 courses (11%) with the 1-h infusion and in 29 courses (28%) with the 3-h infusion. The most frequent reasons for dose delays were incomplete recovery from previous toxicity, administrative reasons and public or patient holidays.

3.2. Toxicity profile

All patients and all treatment cycles were included in the safety evaluation. The most frequently encountered severe non-haematological toxicities attributable to ET-743 were nausea and vomiting, fatigue/asthenia/lethargy/malaise (further referred to as 'fatigue') and biochemical hepatic toxicity (Tables 3 and 4). Grades 3–4 neutropenia and thrombocytopenia were observed at the highest dose level with the 1-h infusion and at dose

Table 1
Patient's characteristics

Demographic variable	1-h infusion	3-h infusion	Total population
No of patients treated	40	32	72
Male/female	20/20	14/18	34/38
Median age in years (range)	57 (24–77)	57 (27–71)	57 (24–77)
Median WHO performance status (range)	1 (0–2)	1 (0–2)	1 (0–2)
Primary tumour site (number of patients)			
Gastrointestinal tract	16	10	26
Lung	3	4	7
Kidney	5	2	7
Ovary	3	2	5
Oesophagus	2	3	5
Cervix	3	–	3
Gastrointestinal stromal tumor	1	2	3
Uterine sarcoma	2	1	3
Sarcomatoid bladder cancer	1	–	1
Other	4[a]	5[b]	9[a,b]
Unknown	–	3	3
Prior treatment (number of patients)			
Surgery	38	30	68
Radiotherapy	13	8	21
Chemotherapy	35	26	61
1 line	13	9	22
2 lines	13	8	21
3 or more lines	9	9	18
Radiotherapy and chemotherapy	13	7	20

WHO, World Health Organization.
[a] Breast, melanoma, pancreas, peritoneum.
[b] Bladder, breast, liver, melanoma, pancreas.

Table 2
Dose escalation steps and distribution of patients and treatment cycles over the dose levels studied

Step		ET-743 dose ($\mu g/m^2$)	No. patients	No. cycles
1 h	3 h			
1	–	50	3	13
2	–	100	3	10
3	–	200	3	16
4	–	330	3	10
5	–	440	3	9
6	–	585	5	20
7	–	800	6	13
8	–	1100	6	13
9	–	1000	8	20
–	1	1000	3	8
–	2	1300	6	16
–	3	1500	6	19
–	4	1800	4	5
–	5	1650	13	55
Total (1 h/3 h)			40/32	124/103

levels of 1300 $\mu g/m^2$ and above with the 3-h infusion (Table 5). Non-haematological toxicities of grades 3–4 were seen more frequently and sometimes at lower doses than haematological toxicities. The lowest dose level at which Grades 3-4 transaminase increases were seen was 1000 $\mu g/m^2$.

Further adverse events reported in association with the infusion of ET-743 were phlebitis (grades 1–3), stomatitis (grades 1–2), altered taste (grades 1–2), diarrhoea (grades 1–2), anorexia (grades 1–2), fever (grade 2), proteinuria (grades 1–2), hypoalbuminemia (grades 1–2), hyperglycemia (grades 1–2) and constipation (grades 1–2). These were not dose-limiting and no major differences in the pattern of toxicities were observed between the 1-h and the 3-h infusions.

The most frequent reason for cycle delays in the 1 h study was increased transaminases in three out of 84 cycles. In the 3-h study haematological toxicity was the reason for delaying 11 out of 71 cycles; this mainly consisted of neutropenia and, less frequently, thrombocytopenia.

Neutropenia occurred in association with 82 treatment cycles (36%) and was grade 1–2 in most cases (Table 5). Grade 3–4 neutropenia was observed in 15 patients (20%) and occurred only at ET-743 doses of 1100 $\mu g/m^2$ and above. Only 2 patients had dose-limiting myelosuppression, 1 at the 1100 mg/m^2 dose level following a 1-h infusion and the other with a 1800 mg/m^2 given over 3 h. Recovery from neutropenia grades 3–4 was usually within 1–2 weeks, but in two out of two cycles delayed in the 1-h study and 11 out of 29 cycles (4 patients) in the 3-h study, there were extremes up to 26 days.

Table 3
Worst-grade toxicity of ET-743 following 1- and 3-h intravenous (i.v.) infusions; depicted are the numbers of patients (%) per dose level experiencing the specified grade of study drug-related toxicity, excluding events not considered related to the study drug

Dose (μg/m^2)	No. of patients	Nausea and vomiting		Fatigue[a]	
		Grade 3	Grade 4	Grade 3	Grade 4
1-h infusion					
50–585	20	3 (15)	–	1 (5)	–
800	6	2 (33)	–	–	–
1000	8	–	–	–	–
1100	6	3 (50)	–	1 (17)	–
3-h infusion					
1000	3	–	–	–	–
1300	6	3 (50)		1 (17)	–
1500	6	2 (33)		3 (50)	–
1650	13	2 (15)		3 (23)	–
1800	4	1 (25)	1 (25)	1 (25)	–

[a] Including asthenia, lethargy and malaise.

Table 4
Hepatic toxicity of ET-743. Depicted are the number of patients (pt) and cycles (cy) associated with the specified grades of study drug-related toxicity, excluding events not considered related to the study drug

Dose (μg/m^2)	No. patients /no. cycles	Elevated ALT				Elevated alkaline phosphatase				Hyperbilirubinaemia			
		Grades 1–2		Grades 3–4		Grades 1–2		Grades 3–4		Grades 1–2		Grades 3–4	
		Pt	Cy	Pt	Cy	Pt	Cy	Pt	Cy	Pt	Cy	Pt	Cy
1-h infusion													
50–85	20/78	7	13	1	1	2	6	–	–	–	–	–	–
800	6/13	4	7	–	–	1	1	–	–	–	–	–	–
1000	8/20	5	7	5	13	2	5	–	–	–	–	–	–
1100	6/13	3	3	4	6	3	5	–	–	3	4	1	1
3-h infusion													
1000	3/8	2	2	1	1	2	3	–	–	–	–	–	–
1300	6/16	5	11	3	5	2	4	–	–	1	1	–	–
1500	6/19	3	6	5	11	4	5	–	–	1	2	1	1
1650	13/55	9	32	11	20	9	24	2	2	4	4	1	1
1800	4/5	1	2	3	3	1	1	1	1	1	1	1	1

ALT, alanine aminotransferase.

Thrombocytopenia was observed in 44 treatment cycles (19%), but was rarely severe (Table 5). Grade 3–4 thrombocytopenia was noted only in patients treated at doses of 1100 μg/m^2 and above. 2 patients experienced grade 4 thrombocytopenia, 1 at 1100 μg/m^2 by 1-h infusion and 1 at 1800 μg/m^2 given as a 3-h infusion. Severe anaemia was not a feature following either the 1- or 3-h infusions.

Biochemical hepatic toxicity was evident from elevated serum levels of liver enzymes, notably ALT (Table 4) and AST (data not shown; patterns for ALT and AST were similar and ALT is a more specific indicator of liver function). Grades 1–2 elevation of transaminases was a frequent observation, occurring in approximately 40% of all cycles. Grades 3–4 transaminase elevation was frequently reported at the higher dose levels. With the 3-h infusion, 40 cycles (39%) were associated with grades 3–4 elevated transaminases. Serum levels of liver enzymes peaked at a median of three days (range: 3–7 days) after administration of ET-743, with recovery to grade 1 within a median of 7 days to recovery after treatment with ET-743 (range 3–18 days). The median number of days with grades 3–4 elevated transaminase was 5 (range 2–13 days).

Increases in transaminases followed a predictable pattern and values returned to baseline within the timeframe specified in the definition of DLTs in almost all patients. Thus, grades 3–4 increase in transaminases was not dose-limiting, as they recovered by day 28. Elevation of serum alkaline phosphatase and bilirubin was noted, but was rarely grade 3 and there were no grade 4

Table 5
Haematological toxicity of ET-743. Depicted are the number of patients (pt) and cycles (cy) associated with the specified grades of study drug-related toxicity, excluding events not considered related to the study drug

Dose (μg/m^2)	No. patients/ no. cycles	Neutropenia				Thrombocytopenia				Anaemia			
		Grades 1–2		Grades 3–4		Grades 1–2		Grades 3–4		Grades 1–2		Grades 3–4	
		Pt	Cy	Pt	Cy	Pt	Cy	Pt	Cy	Pt	Cy	Pt	Cy
1-h infusion													
50–585	20/78	–	–	–	–	1	1	–	–	10	18	1	1
800	6/13	2	2	–	–	1	1	–	–	3	6	–	–
1000	8/20	1	5	–	–	1	1	–	–	3	5	1	1
1100	6/13	3	4	2	2	1	1	1	1	5	6	–	–
3-h infusion													
1000	3/8	–	–	–	–	–	–	–	–	2	3	–	–
1300	6/16	4	10	1	1	1	2	–	–	5	11	–	–
1500	6/19	1	3	2	9	3	6	1	1	6	16	–	–
1650	13/55	12	25	8	17	4	23	4	5	11	43	1	1
1800	4/5	1	2	2	2	1	1	1	1	4	5	–	–

cases (Table 4, Fig. 1). This toxicity did not appear to be cumulative.

Initially, prophylactic antiemetic treatment was not used, but since many patients experienced nausea and vomiting, prophylactic treatment with domperidone, or $5HT_3$-antagonists if domperidone proved inadequate, was instituted. During the 3-h infusion phase of the study, prophylaxis and treatment of emesis with dexamethasone was permitted. Despite the use of anti-emetic prophylaxis nausea and vomiting were reported in most cycles in all except 2 patients with the 3-h infusion. In 50% of patients, vomiting occurred on days 2–5, with a median duration of 1 day.

Phlebitis was observed in several patients during the initial dose escalation steps. The infusion volume was, therefore, doubled from 250 to 500 ml from dose level 7 (800 μg/m^2), and in the initial patients receiving the 3-h infusion. The final 12 patients, received ET-743 at a dose of 1650 μg/m^2 in a volume of 1000 ml. Despite these precautions, phlebitis grades 1–3 was frequently observed in 16 of 23 patients who received ET-743 as a 3-h infusion via a peripheral line (grade 1 in 1 patient, grade 2 in 14 patients, and grade 3 in 1 patient); the remaining patients received ET-743 through a central line.

Most patients reported fatigue. In 20 patients, this was pre-existing, but worsened during treatment with ET-743. 14 patients, mostly at doses of 1300 μg/m^2 or higher, reported grade 3 fatigue and this was considered related to ET-743 in 10 of them.

3.3. MTD and the recommended dose

With the 1-h infusion schedule, DLTs were observed in 3 of 6 patients receiving 1100 μg/m^2 (Table 6), which

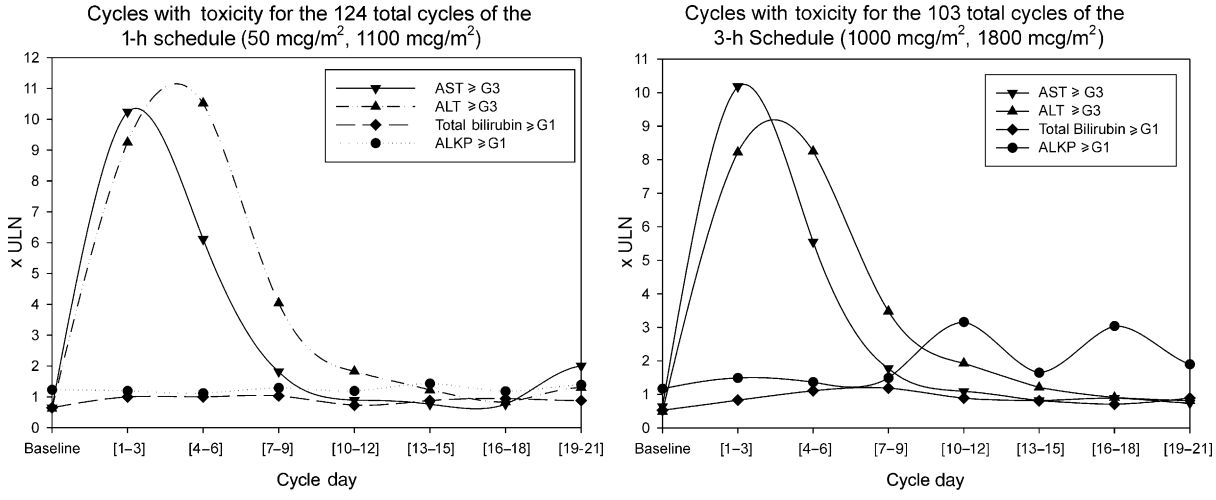

Fig. 1. Time course of liver biochemistry tests after treatment with ET-743. ULN, upper limit of normal; G, grade; ALKP, alkaline phosphatase.

Table 6
MTD, DLTs and recommended dose for phase II studies

Infusion duration	MTD		Recommended phase II dose	
	Dose ($\mu g/m^2$)	DLTs	Dose ($\mu g/m^2$)	DLTs
1 h	1100	– Fatigue (grade 3) in 1/6 patients – Neutropenia >5 days (grade 4) in 1/6 patients – Thrombocytopenia (grade 4) in 1/6 patients	1000	
3 h	1800	– Fatigue (grade 3) in 1/4 patients – Thrombocytopenia (grade 4) and bilirubinaemia (grade 3) in 1/4 patients	1650	– Thrombocytopenia (grade 3) and neutropenia with infection (grade 3) in 1/13 patients

MTD, maximum tolerated dose; DLT, dose-limiting toxicity.

we defined as the MTD. The dose level below the MTD (1000 $\mu g/m^2$) was well tolerated and would, therefore, be the recommended dose for phase II studies with a 1-h infusion of ET-743.

Using the 3-h infusion, the MTD was 1800 $\mu g/m^2$ defined by grade 3 fatigue and grade 4 thrombocytopenia with grade 3 bilirubinaemia as the DLTs (Table 6). At the 1650 $\mu g/m^2$ dose level, only one DLT was noted in a total of 13 patients entered. Therefore, this was the recommended dose for phase II trials of ET-743 given as a 3-h i.v. infusion.

3.4. Antitumour activity

49 patients (68%) were evaluable for tumour response, 27 with the 1-h infusion and 22 with the 3-h infusion. Response could not be evaluated in the remainder due to the absence of a measurable lesion at baseline ($n=8$), lack of repeat tumour measurements ($n=13$), baseline measurement more than 2 months prior to start of treatment ($n=1$) or the patient going off study prematurely ($n=1$). The data for both infusion schedules combined, showed PD in 33 patients and SD in 14 patients. Two objective tumour responses (one CR and one PR) were observed, both with the 3-h infusion schedule.

The patient with a CR was a 48-year old female with a leiomyosarcoma of the uterus, that had metastasised to the lungs. She received 10 cycles of ET-743 at a dose of 1500 $\mu g/m^2$ and had a CR of 18 weeks duration and a total response duration of 37 weeks. The PR was observed in a 51-year-old female with a stromal sarcoma of the colon, metastasised to the liver, who received 17 cycles of treatment at 1650 $\mu g/m^2$. After cycle 13, she had a PR of 6 months duration. In addition, a 24-year old female with a histologically-confirmed metastatic melanoma treated with 585 $\mu g/m^2$ ET-743 as a 1-h infusion had a pathological CR when the persisting lymph nodes were excised, showing no remaining tumour cells, at the end of treatment and remains disease-free after 3 years; her best objective clinical response was recorded as SD. Another 67-year-old man with a gastric stromal sarcoma had a 37% reduction in tumour size and SD for 20 weeks. Several other patients showed long-lasting SD up to 20–28 weeks. Additionally, 9 patients at all dose levels with colorectal, mesothelioma, bile duct carcinoma and bladder cancer had SD which lasted for 6 or more cycles of ET-743.

3.5. Pharmacokinetics

Pharmacokinetic parameters of ET-743 could be calculated for 44 patients, 13 at the higher dose levels of the 1-h infusion and 31 with the 3-h infusion (Table 7). In a number of patients, pharmacokinetic samples were also collected during the second treatment cycle (see Ref. [20] for a detailed account).

Values for the clearance (CL) of ET-743 with the 1-h infusion schedule suggested non-linear pharmacokinetics. However, with the 3-h infusion schedule CL did not significantly vary with the dose of ET-743 administered ($r=-0.07$, $P=0.70$), whereas the area under the plasma concentration versus time curve (AUC) showed a linear increase with dose ($r=0.18$, $P=0.02$). There was considerable interpatient variability in the pharmacokinetic parameters in this study as illustrated by a 14–69% interpatient variability in the AUC values. Mean plasma concentrations at the recommended phase II dose (1650 $\mu g/m^2$ as a 3-h infusion) were in the nanomolar range for a number of hours during and after the drug administration and remained above 0.2 nM (0.15 ng/ml) for the remainder of the 24-h sampling period (Fig. 2).

In a full analysis of the pharmacokinetic data obtained with the 3-h infusion schedule, [20] grade 3–4 biochemical hepatotoxicity was significantly associated with higher C_{max} values during the first cycle ($P=0.02$ for alkaline phosphatase and $P=0.04$ for AST; data not shown).

Table 7
Pharmacokinetic parameters of ET-743 during the first treatment cycles

Dose (μg/m^2)	N	AUC (h ng/ml)	CL (l/h)	C_{max} (ng/ml)	$t_{1/2}$ (h)	V_{ss} (l)
1-h infusion[a]						
800	4	23 (±7.5)	58 (±25)	13 (±6.9)	50 (±63)	2000 (±2500)
1000	5	36 (±6.4)	32 (±6.1)	17 (±5.2)	33 (±14)	910 (±720)
1100	4	52 (±16)	25 (±9.0)	18 (±4.5)	36 (±13)	980 (±800)
3-h infusion						
1000	3	31 (±16)	66 (±29)	5.1 (±1.2)	46 (±31)	2200 (±1300)
1300	6	25 (±8)	100 (±26)	5.4 (±1.7)	28 (±19)	2000 (±1600)
1500	6	59 (±29)	51 (±24)	10 (±3.7)	43 (±42)	1500 (±1600)
1650	12	38 (±10)	87 (±30)	8.6 (±2.5)	26 (±7)	1400 (±490)
1800	4	71 (±38)	55 (±30)	12 (±6.8)	31 (±14)	1500 (±1200)

CL, clearance; C_{max}, maximum concentration; AUC, area under the plasma concentration versus time curve; $t_{1/2}$, half-life; V_{ss}, steady state volume. Values are presented as means (±standard deviation).

[a] Data at the lower dose levels were not analysed due to a lack of appropriate limit of quantitation.

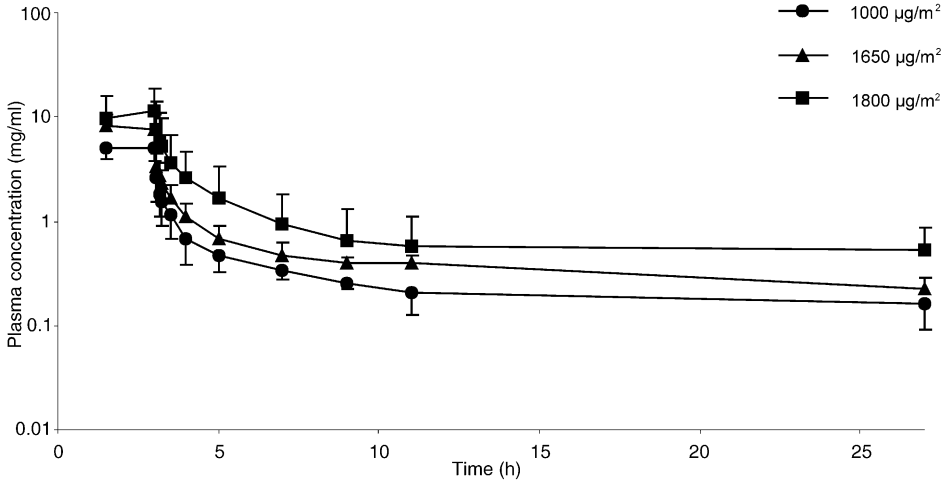

Fig. 2. Plasma concentrations versus time profile of ET-743 during the first cycle of treatment in patients treated with 1000 μg/m^2 (N=3), 1650 μg/m^2 (N=12), or 1800 μg/m^2 (N=4) using the 3-h infusion schedule. Mean values (±standard deviation) are depicted.

4. Discussion

This phase I trial shows that ET-743 can be administered safely by 1- and 3-h i.v. infusions to patients with solid tumours. The MTD and the recommended phase II dose of the drug could be increased markedly from 1100 and 1000 μg/m^2 to 1800 and 1650 μg/m^2, respectively, by prolonging the duration of the infusion from 1 to 3 h, without affecting the toxicity profile. The MTD for the 3-h infusion in this study was identical to the MTD found in another phase I study investigating ET-743 using a 24-h infusion [15]. The recommended dose for phase II in the latter study was 1500 μg/m^2. The toxicity profiles observed in both phase I studies were very similar with haematological and hepatic toxicity constituting the most prominent toxicities. Our study demonstrates, therefore, the feasibility of treating patients with ET-743 as a 3-h infusion without compromising the total dose administered compared with a 24-h infusion. Since the latter would require hospitalisation of the patients, the 3-h infusion should be considered the preferred option for practical reasons.

Comparison of our data with those of the recently published phase I study of ET-743 at the d×5 schedule indicates that no improvement in tolerability can be achieved by fractionating the dose [18]. The MTD and the recommended phase II dose in the latter study were 1900 μg/m^2 per cycle and 1625 μg/m^2 per cycle, respectively. DLTs in the d×5 study were neutropenia, thrombocytopenia and rhabdomyolysis. Similarly, prolonged infusion of ET-743 over 72 h did not allow the administration of a higher total dose per cycle. The MTD and recommended dose in this study were 1200 and 1050 μg/m^2, respectively, while rhabdomyolysis, increased transaminases (which was considered a DLT in that study), neutropenia and thrombocytopenia were DLTs [17].

The 3-h infusion schedule has been implemented successfully in an ongoing phase II programme of ET-743 in a variety of tumour types, although the dose has been reduced to 1500 or 1300 μg/m^2 to enhance the safety of this schedule. The initial phase II patients almost invariably developed grade 3–4 elevated transaminases and grade 2–4 asthenia at 1650 μg/m^2 as a 3-h infusion. Furthermore, four cases of febrile neutropenia were noted among the first 26 patients at this dose and schedule.

There was a remarkable resemblance between the toxicity profiles of ET-743 in our study and in other completed phase I studies. Most of the major toxicities observed in patients, i.e. haematological toxicity, hepatic toxicity, and nausea and vomiting, could be predicted from preclinical toxicity studies. Toxicities of ET-743 were generally manageable. Neutropenia was usually uncomplicated and reversible within a limited time-frame. Liver toxicity was evident in many patients from marked increases in serum ALT and AST. Signs of cholestasis, as evidenced by increases in alkaline phosphatase and bilirubin, were apparent, although these occurred less frequently and were of a lower grade than the increases in transaminases. Elevation of plasma transaminases followed a highly predictable pattern with an acute onset and rapid recovery, but without affecting patients' well being. Grades 3–4 elevations in transaminases were, therefore, not considered dose-limiting in this study nor in other phase I studies of ET-743 [15,18].

Despite the use of antiemetic prophylaxis nausea and vomiting were reported in most cycles in all, except 2 patients with the 3-h infusion. Fatigue was significant in the 3-h study at the recommended dose (1650 μg/m^2) and also at the dose level below this (1500 μg/m^2).

The high incidence of phlebitis at the site of infusion is such that the use of a central line should be strongly considered in future studies. Of note, renal failure, rhabdomyolysis, mucositis, alopecia and diarrhoea were not seen in this trial.

The pharmacokinetic analyses, conducted as part of this study, demonstrated linear kinetics for the 3-h infusion schedule, with considerable interpatient variability in the AUC values. Pharmacologically relevant plasma concentrations of ET-743, based on previous *in vitro* antitumour studies [1,2], were achieved and maintained for several hours during and after the infusion of the recommended phase II dose of 1650 μg/m^2 as a 3-h infusion. Pharmacokinetic-pharmacodynamic relationships could be delineated for the main haematological and hepatic toxicities of ET-743 in this study. Both toxicities appeared to correlate with exposure to ET-743. Haematological toxicity correlated with AUC, dose and C_{max}. The risk of severe grades 3–4 hepatotoxicity increased at higher C_{max} values. Similar conclusions were reached in the phase I study of ET-743 given as a 24-h infusion which showed that the grade of transaminase elevation and the likelihood of severe haematological toxicity correlated with both the dose and AUC of ET-743 [15,16].

It is noteworthy that objective tumour responses and the 37% reduction in tumour size in our study were seen in patients with sarcomas. In other phase I studies of ET-743, several tumour responses have also been noted in patients with sarcomas, which is consistent with the strong cytotoxic effect noted in a study of human cancer cell lines, where ET-743 exhibited extremely potent activity against several soft-tissue sarcoma cell lines, with IC_{50} values well below 1 pM [1]. These observations led to the initiation of phase II studies of ET-743 in a range of sarcomas, that have already confirmed the clinical activity of ET-743 [24,25]; phase II studies have also been carried out in other solid tumours, such as ovarian and breast cancer, where ET-743 has shown interesting preliminary evidence of activity [26,27].

In conclusion, this phase I study demonstrates that ET-743 can be administered safely to patients with metastatic or advanced solid tumours as a 1-h and as a 3-h i.v. infusion. The MTD with the 1-h infusion is lower than that of the 3-h infusion. The latter schedule allowed the administration of the same maximum dose as a 24-h infusion in another phase I study [15], and a similar pattern of haematological and biochemical hepatic toxicities were observed. Our phase I study, as well as other phase I studies of ET-743, provides preliminary evidence of efficacy in patients with soft-tissue sarcoma.

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