Cancer Chemother Pharmacol (2011) 68:1397–1403
DOI 10.1007/s00280-011-1612-1

ORIGINAL ARTICLE

Spisulosine (ES-285) given as a weekly three-hour intravenous infusion: results of a phase I dose-escalating study in patients with advanced solid malignancies

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Received: 23 December 2010 / Accepted: 4 March 2011 / Published online: 5 April 2011

Abstract
Purpose Spisulosine is a marine compound that showed antitumor activity in preclinical studies. We report results of a phase I trial performed in patients with advanced solid tumors with the marine compound, with the aim to determine the maximum tolerated dose (MTD) of a weekly 3-h intravenous (iv.) infusion, and to evaluate the safety, efficacy, and pharmacokinetics (PK) of the compound.
Patients and methods Two centers contributed 25 patients to the trial, and 7 dose levels were explored.
Results In dose levels ranging from 4 to 128 mg/m^2/day, no dose-limiting toxicities (DLT) were observed. One patient had DLT at 200 mg/m^2, a reversible grade 3 ALT increase. The MTD was not reached due to early termination of the Spisulosine trial program but is considered to be likely in the range of 200 mg/m^2 for this schedule. Drug-related adverse reactions included mild to moderate nausea, pyrexia, injection site reactions, and vomiting. One case of grade 4 peripheral motor and sensory neuropathy associated with general weakness and pain was observed during treatment cycle 4 and possibly contributed to the death of the patient. Grade 3 laboratory abnormalities included anemia and lymphopenia and increases in liver enzymes (alkaline phosphatase, transaminases, and bilirubin). Objective responses were not observed, and only four patients had short-lasting stable disease (<3 months). The PK data indicated a wide distribution, a long residence time, and dose proportionality of the agent.
Conclusions Hepato- and neuro-toxicity are schedule independent dose-limiting adverse events for this marine compound, as illustrated by this and other early clinical trials.

Keywords ES-285 · Spisulosine · Phase I · Marine compounds · Pharmacokinetics · Hepatotoxicity · Neurotoxicity

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Introduction

Spisulosine (ES-285, PM95118, IL0111) is the analog of a phospholipid sphingosine, which was isolated from the marine mollusk *Spisula polynyma* [1] (Fig. 1, 2). Preclinical studies indicated strong antineoplastic potential against a variety of malignancies, ranging from hematological malignancies to solid tumors [1–3]. The mechanism of action of Spisulosine, which induces programmed cell death in vitro, is still speculative. GTP binding protein regulating actin stress fibers has been proposed as a potential molecular target, as tumor cells exposed to the agent show suggestive phenotypic changes and expression profiling data indicated modulation of genes related to the actin cytoskeleton [4]. Spisulosine seems also to be capable of activating caspases 3 and 12, the poly ADP-ribose

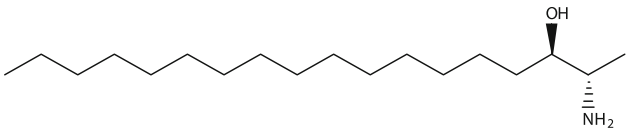

Fig. 1 Chemical structure of Spisulosine (molecular formula $C_{18}H_{39}NO$)

Fig. 2 Spisula polynyma (Alaska)

polymerase pathway, and p53 phosphorylation [5]. Four different administration schemes of single-agent Spisulosine have been assessed in separate clinical phase I studies [6–9]. The current trial evaluated a 3-h infusion given i.v. every week in 3-week intervals, with the objective to identify the MTD, to establish the safety profile of this schedule, to assess preliminary signs of antitumor activity, and to assess the PK profile of this novel compound.

Patients and methods

Patient selection

Patients had a histologically confirmed solid tumor and had exhausted standard treatment options, were 18–70 years old, had no acute adverse effects from prior treatments, a life expectancy ≥12 weeks, measurable or non-measurable disease according to RECIST [10], Eastern Cooperative Oncology Group performance status of 0–2 and good organ function (creatinine <1.5 mg/dl or calculated creatinine clearance >60 ml/min; alanine aminotransferase [ALT] and aspartate aminotransferase [AST] ≤2.5 × upper limit of normal [ULN]; bilirubin ≤1.5 × ULN); absolute neutrophil count [ANC] ≥1.5 × 10^9/l, platelets ≥100 × 10^9/l); left ventricular ejection fraction ≥50%).

In case of clinically relevant preexisting comorbidity, increased cardiac risk, brain metastasis or if they had received chemotherapy, radiotherapy or biological therapy within 4 weeks or immunosuppressors within 3 weeks previous to enrollment, patients were excluded from trial participation. Pregnant or lactating women were not entered. All patients provided written informed consent, and ethics committee approval was obtained in both involved clinical sites.

Study design

Patients were recruited from centers in Belgium (University Hospital Gasthuisberg, Leuven) and in the Netherlands (Free University Hospital, Amsterdam). Spisulosine, supplied as 50 mg of lyophilized powder by PharmaMar S.A., Spain, was reconstituted in 5 ml of water and made up to 250 ml with 5% dextrose and given weekly as a 3-h i.v. infusion for at least one three-week cycle [11, 12]. Dose escalation was performed in subsequent cohorts of 3 to a maximum of 6 patients. The starting dose was 4 mg/m^2/day (total dose of 12 mg/m^2 per cycle), corresponding to 30% of the MTD in mice. Interpatient dose escalation up to 200 mg/m^2/day was considered by protocol. The dose was doubled per cohort until dose-limiting toxicity (DLT) was observed. The DLT window was the first 6 weeks of treatment (first two three-week cycles), and DLT was defined as a treatment delay >2 weeks, ANC <0.5 × 10^9/l for >5 days or <1.0 × 10^9/l with fever, platelets <25 × 10^9/l, a relative decrease in LVEF ≥20% compared to the pre-study assessment, or any other grade 3/4 non-hematological adverse event except for non-adequately treated nausea/vomiting. In the absence of DLT during the first two treatment cycles, three patients were treated per dose level. A 3-week safety interval was mandated by protocol before the second and third patient of a cohort could start treatment. If DLT occurred, up to three additional patients were treated. If two or more experienced DLT, this dose level was considered the MTD for this schedule. The recommended dose for further clinical studies was defined as the highest dose level where DLT was observed in less than two out of six trial participants.

Clinical assessments

Routine medical examinations were performed at the start of every cycle and before administering the weekly drug infusion. Electrocardiogram, blood counts, and serum chemistry including cardiac enzymes were performed weekly. Tumor response was evaluated every 6 weeks according to RECIST [10], adverse events were scored continuously following the National Cancer Center Common Toxicity Criteria (NCI-CTC) v. 2.0.

Pharmacokinetics

Heparinized blood samples (5 ml) were collected according to protocol and analyzed centrally with a validated high-performance liquid chromatography system coupled with electrospray ionization tandem mass spectrometry (1.0 ng/ml limit of quantification) [13].

WinNonlin software (version 5.0.1) with standard non-compartmental methods was used to calculate the area under the plasma concentration curve from time 0 to the last quantifiable concentration [AUC_{last}], peak plasma concentration (C_{max}), total body clearance (CL), terminal half-life ($t_{1/2}$), and volume of distribution (Vz), and the statistical analysis was done with SAS® version 8.2 (SAS Institute, Cary, NC).

Results

Patient characteristics

Twenty-five patients were treated between May 2003 and November 2005, with 23/25 being evaluable for the safety and efficacy endpoints of the trial. Patient characteristics are shown in Table 1. Malignant melanoma ($n = 5$ patients), colorectal cancer ($n = 4$), prostate cancer ($n = 2$), and chondrosarcoma ($n = 2$) were the most common entities. All patients had metastatic disease at study entry, and all but one had received prior chemotherapy (96%), with a median of three lines per patient (range, 1–10).

Table 1 Patient characteristics

Characteristics	*N*
No. of patients	25
Male/female	17/8
Median age in years (range)	54 (21–74)
ECOG performance status 0/1/2	9/14/2
Primary cancer	
Melanoma	5
Colorectal	4
Chondrosarcoma	2
Prostate	2
Other ($n = 1$/tumor type)	12
Metastatic	25
Median *N* of involved sites (range)	3 (1–6)
Prior therapy	
Chemotherapy	24
Median *N* of lines (range)	3 (1–10)

ECOG Eastern cooperative oncology group

Dose escalation and DLTs

All 7 dose levels planned according to protocol were visited, and doses were escalated until 200 mg/m^2/day (Table 2). Sixty-one cycles were administered with a median of 2 cycles per patient (range, 1–5). Treatment was discontinued due to disease progression ($n = 22$ patients), adverse events ($n = 2$), or withdrawal of consent ($n = 1$). The median cumulative administered dose was 160 mg/m^2 (range, 8–2,400 mg/m^2), with a median relative dose intensity of 83% (range, 33–102%).

One patient experienced a DLT, a reversible grade 3 increase in serum ALT in the first cycle at the highest dose level of 200 mg/m^2/day (Table 2). One patient experienced grade 4 sensory and motor neuropathy during cycle 4, which possibly contributed to the patient's subsequent death. An autopsy was not performed. MTD was not formally reached due to early study termination, following a consensual agreement between the sponsor and investigators involved in the Phase I program of Spisulosine, based on fatal neurotoxicity experienced by a patient in a parallel trial, the observation of grade 4 neuropathy during consecutive treatment cycles of patients in our trial and limited evidence of antitumor activity in this and other studies. The MTD is likely in the range of 200 mg/m^2/day for the weekly 3-h schedule, with one DLT observed in cycle 1 in this prematurely closed study.

Overall tolerability

The most frequent adverse events were nausea, pyrexia, injection site reactions/phlebitis, and vomiting (20, 20, 20, and 16% of patients, respectively; Table 3). All drug-related AEs were mild (grade 1 or 2), with the exception of a patient who developed grade 4 peripheral motor and grade 4 sensory neuropathy during cycle 4 of treatment.

The most frequent hematological events were anemia (all patients, 20% with grade 3) and lymphopenia (72% of patients, 20% with grade 3). The most common biochemical abnormalities were increases in serum alkaline phosphatase and transaminase levels. The incidence of other biochemical abnormalities was low. Three patients had grade 3 AST increases, and 2 patients had grade 3 ALT increases, one of these events qualifying as the only DLT observed in this trial.

Drug-related serious adverse events included one patient with pyrexia and one patient with neuropathy. The first patient was a 61-year-old male with metastatic colorectal cancer who developed fever up to 40 degrees Celsius after the first administration of Spisulosine 32 mg/m^2; he recovered without sequelae and received further infusions without similar complications. The second patient was a 69-year-old male with metastatic melanoma who received

Table 2 Dose escalation and dose-limiting toxicity for Spisulosine

Dose level (mg/m^2/day)	No. of patients	No. of cycles, median (range)	Days on study, median (range)	No. of patients with DLT
4	3	2 (2–2)	27 (26–42)	0
8	5	2 (1–3)	27 (7–49)	0
16	3	2 (2–4)	28 (28–70)	0
32	3	2 (2–2)	28 (28–28)	0
64	3	4 (2–5)	70 (28–92)	0
128	3	3 (2–4)	49 (29–77)	0
200	5	2 (1–5)	28 (7–91)	1
Total	25	61	28 (7–92)	1

Total number of cycles = 61

DLT Dose-limiting toxicity

Table 3 Grade 3 treatment-emergent toxicities per patient

Dose level (mg/m^2)	No. of patients treated	No. of patients					
		Anemia	Lymphopenia	AST increase	ALT increase	AP increase	Bilirubin increase
4–32	14	3 (21%)	3 (21%)	–	–	3 (21%)	2 (14%)
64	3	1 (33%)	1 (33%)	1 (33%)	1 (33%)	1 (33%)	–
128	3	–	–	–	–	–	–
200	5	1 (20%)	1 (20%)	2 (40%)	1 (20%)	–	1 (20%)
Total	25	5 (20%)	5 (20%)	3 (12%)	2 (8%)	3 (12%)	3 (12%)

In addition to these grade 3 laboratory events, a case of grade 4 peripheral motor neuropathy and grade 4 peripheral sensory neuropathy was observed in one patient in the 128 mg/m^2 dose cohort

Grade 1/2 clinical adverse events included injection site reactions (20% of all patients), pyrexia (20%), nausea (20%), vomiting (16%), asthenia (12%), myalgia (8%), limb pain (8%), back pain (4%), dysgeusia (4%), headache (4%), paresthesia (4%), and hot flushes (4%)

Table 4 Summary of pharmacokinetic parameters on day 1

Dose (mg/m^2/day)	No. of evaluable patients	C_{max} (ng/ml)	AUClast (h ng/ml)	AUC (h ng/ml)	CL (L/h)	Vz (L)	$t_{1/2}$ (h)
4	2	9.3 (17.4)	24.3 (5.2)	31.7 (33.2)	258.4 (34.6)	1,521.5 (114.9)	5.2 (124.7)
8	5	68.0 (95.7)	204.5 (59.8)	302.1 (70.7)	92.8 (93.4)	2,554.3 (93.1)	41.2 (94.4)
16	3	41.1 (8.4)	170.0 (28.4)	272.5 (38.1)	113.0 (40.6)	7,152.3 (14.6)	50.8 (51.3)
32	3	58.9 (32.3)	167.4 (31.3)	171.4 (30.9)	400.0 (43.1)	1,205.6 (47.1)	2.1 (4.0)
64	3	168.0 (1.6)	522.6 (25.2)	575.5 (30.3)	224.5 (33.9)	7,576.5 (99.7)	32.2 (123.8)
128	3	379.7 (18.1)	1,292.8 (11.5)	1,416.2 (13.5)	169.2 (22.4)	11,299.0 (95.2)	48.7 (103.3)
200	4	658.0 (12.5)	2,926.2 (54.1)	3,287.6 (57.9)	142.4 (40.9)	13,462.5 (78.6)	60.9 (58.5)

Values are mean (coefficient of variation, %)

AUC Area under the plasma concentration–time curve, *CI* confidence interval, *CL* clearance, C_{max} maximum plasma concentration, $t_{1/2}$ terminal phase half-life, *Vz* volume of distribution

8 infusions of the agent at a dose of 128 mg/m^2. He developed grade 1 numbness of the face, hands, and feet prior to the eighth weekly infusion; his symptoms worsened rapidly with the occurrence of neuropathy, pain, and general weakness to grade 4, which ultimately also contributed to his subsequent death according to the investigator's assessment. The patient had no other known risk factors for neuropathy, had no relevant comorbidities and did not receive any other drug associated with potential neurotoxicity. The sensor and motor neuropathy of this patient did not respond to treatment with vitamin B12 or pregabalin. At time of onset of the neuropathy, he had no evidence of disease progression, but the best overall response to spisulosine was progressive disease.

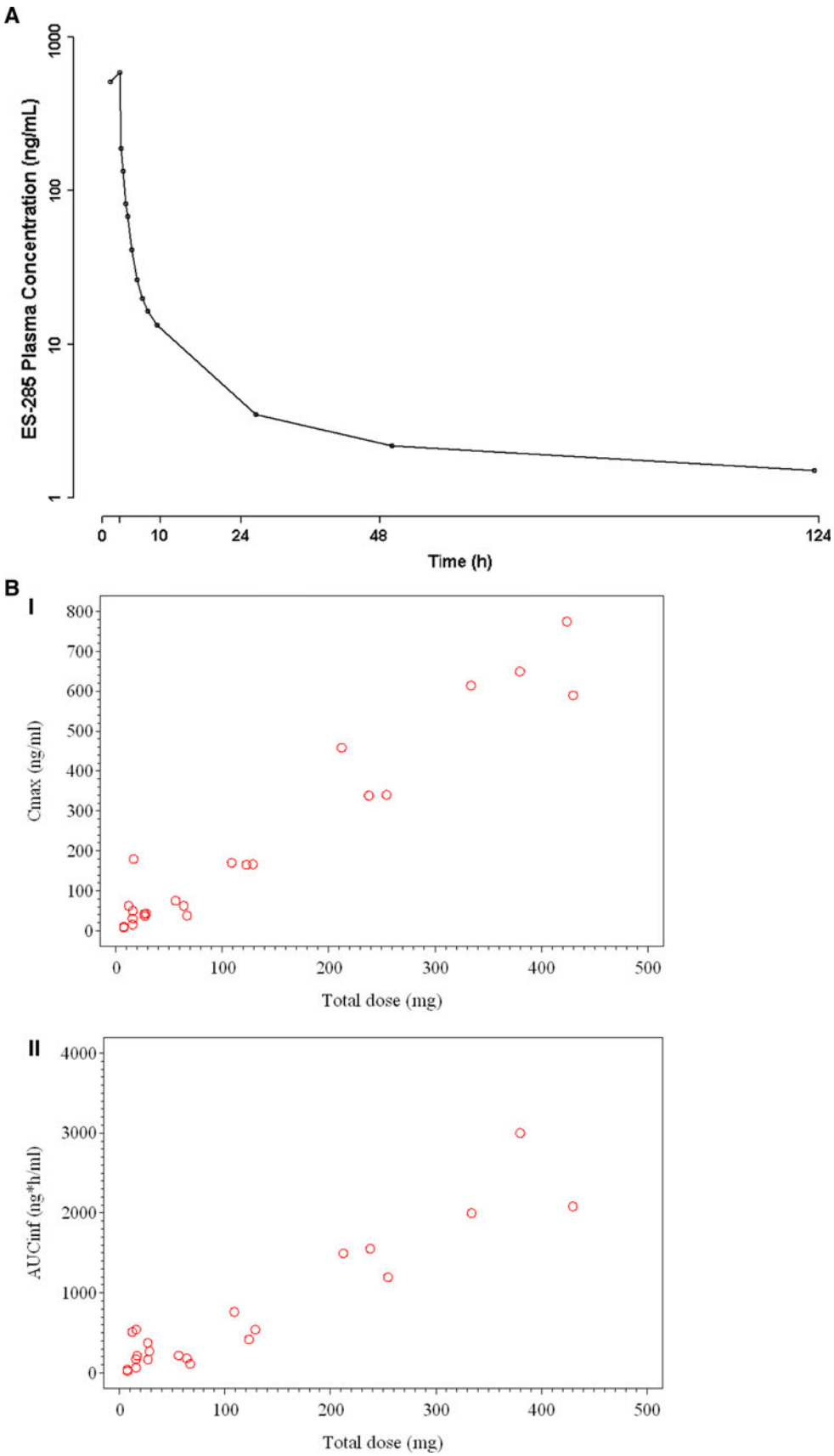

Fig. 3 **a** Representative concentration-time plot for Spisulosine administered on day 1 as a 3-h intravenous infusion (200 mg/m^2). **b** (I) Peak plasma concentration (C_{max}) versus total dose; (II) Area under the curve versus total dose

Antitumor activity

No objective responses were seen among twenty-three evaluable patients. Four patients (two patients with melanoma, and one each with ovarian cancer and chondrosarcoma) showed stable disease, lasting for less than 3 months.

Pharmacokinetics

Twenty-three patients were evaluated for PK (Table 4), which was characterized by a wide distribution (dose-dependent median volume of distribution [V_{SS}] ranging between 1,638 and 6,505 liters) and a long body residence with a median $t_{1/2}$ of 61 h (range, 28–94 h). PK indicated dose linearity, but the sample size was too limited to draw definitive conclusions (Table 4; Fig. 3a, b).

Discussion

Spisulosine, a compound of marine origin, which has antitumor activity in preclinical models [1, 6], was studied as a weekly 3-h i.v. infusion in doses ranging from 4 to 200 mg/m^2. This schedule was generally well tolerated, as illustrated by the low incidence of drug-related grade 3/4 or serious adverse events. Of note, three patients had grade 3 AST increases, and 2 patients had grade 3 ALT increases, one of these adverse drug reactions qualifying as the only DLT observed in this trial. These safety findings are consistent with preclinical investigations and other early clinical studies [6–9], with reversible transaminase increases reported at the highest doses in concurrent phase I clinical trials with Spisulosine using 3 and 24-h i.v. infusions every 3 weeks.

As was the case with the 24-h every 3-week schedule, infusion site reactions were relatively common in this study but did not require dose or schedule modifications. Preclinical toxicity studies suggested that cardiotoxicity could be dose limiting for this compound, but this was not confirmed in our clinical series. Overall, the toxicity profile was consistent between the different administration schedules, with notable toxicities being mainly hepatic and neurological.

Drug-related neurotoxicity has been a common feature in a number of clinical studies with various compounds of natural origin and especially with some marine agents [14, 15]. The neurological events observed in the Phase I program of Spisulosine include cases of sensory or motor neuropathy, neuropathic pain, dizziness, apraxia, aphasia, headache, somnolence, tremor, and decreased level of consciousness. One patient treated with Spisulosine 200 mg/m^2 as a 24-h i.v. infusion developed grade 3 drug-related central neurotoxicity, which led to the patient's death 6 days after the first administration of the experimental agent [6]. The neurotoxic potential of the drug was confirmed in our study. Of note, the neurotoxicity of Spisulosine was described as being more acceptable in another Phase I trial [7]. The pathophysiology of the neurotoxicity of the agent is currently unknown.

The PK profile of Spisulosine in this schedule showed a wide distribution and a long terminal half life, with no evidence of clinically relevant accumulation and a bi-exponential elimination phase. There was an apparent linear relationship between Spisulosine dose and exposure according to C_{max} and AUC.

Recruitment of patients to Spisulosine clinical trials was discontinued during exploration of the last dose level in this study, due to an unfavorable risk/benefit balance, and due to the absence of clinically meaningful antitumor efficacy in the whole clinical program. At that time, only short-lasting stable disease had been reported as efficacy signal from these trials. The seriousness of the neurological toxicity experienced by one patient [6] supported this decision.

In our trial, further patient recruitment and the expansion of the recommended dose cohort were not implemented for ethical reasons. The MTD for this schedule can be estimated to be 200 mg/m^2/day or higher. There was no convincing preliminary evidence of clinical antitumor efficacy.

In conclusion, hepato- and neurotoxicity are likely dose limiting for Spisulosine in the weekly 3-h schedule, which is in line with the results of other phase I trials and led to discontinuation of the clinical development of this marine compound.

Acknowledgments We are grateful to the patients who participated in this trial, the many clinical research associates, nurses, and data managers for their help in patient recruitment, data management, and patient care during the conduct of this trial. This work was supported by PharmaMar SA, Spain.

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