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Phase I safety, pharmacokinetic, and pharmacogenomic trial of ES-285, a novel marine cytotoxic agent, administered to adult patients with advanced solid tumors

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Abstract

A dose-escalation, phase I study evaluated the safety, pharmacokinetics, pharmacogenomics, and efficacy of ES-285, a novel agent isolated from a marine mollusc, in adult cancer patients. Patients received a 24-hour i.v. infusion of ES-285 once every 3 weeks until disease progression or unacceptable toxicity. The starting dose was 4 mg/m^2. Dose escalation in cohorts of at least three patients proceeded according to the worst toxicity observed in the previous cohort. Twenty-eight patients were treated with 72 courses of ES-285 across eight dose levels. No dose-limiting toxicities were seen between 4 and 128 mg/m^2. Two of four patients treated at 256 mg/m^2 had dose-limiting reversible grade 3 transaminitis; one patient at 256 mg/m^2 also had transient grade 3 central neurotoxicity. One of three patients subsequently treated at 200 mg/m^2 died following drug-related central neurotoxicity. Other toxicities included phlebitis, nausea, fatigue, and fever. Pharmacokinetic studies indicated dose proportionality with high volume of distribution (median V_{ss} at 256 mg/m^2 was 2,389 liters; range, 1,615–4,051 liters) and long elimination half life (median $t_{1/2}$ at 256 mg/m^2 was 28 h; range, 21–32 h). The three patients with dose-limiting toxicity had the highest drug exposure. Pharmacogenomic studies of paired surrogate tissue samples identified changes in gene expression following treatment that correlated with increasing dose. Disease stabilization for 6 to 18 weeks was recorded in nine patients. Using this schedule, 128 mg/m^2 was considered safe and feasible. At this dose, pharmacologically relevant concentrations of the drug were safely achieved with pharmacogenomic studies indicating changes in the expression of genes of potential mechanistic relevance. [Mol Cancer Ther 2009;8(6):1430–37]

Introduction

ES-285, a novel antitumor agent isolated from the marine mollusc *Mactromeris polynyma*, exhibits nanomolar antiproliferative tumor activity against a broad range of human cancer cell lines *in vitro* and *in vivo* (1). ES-285 is COMPARE negative, with a novel profile of activity across the NCI 60-cell line panel, suggesting a possible novel mechanism of action (2). Growth inhibitory IC_{50} values ranging from 20 to 200 nmol/L (mean, 80 nmol/L) were reported for ES-285 against prostate, colon, central nervous system, melanoma, and ovarian cancer cell lines of human origin (3). Mechanistic studies have shown that ES-285 modulates RHO protein and ceramide signaling, effects that are associated with disassembly of actin stress fibers (3–6). Cells treated with ES-285 acquire a fusiform shape and later become rounded without focal adhesions, events which are followed by a G_2-M cell cycle arrest. After 24-hour exposure of cells to pharmacologically relevant concentrations of ES-285, sub-G_1 cell cycle accumulation, internucleosomal DNA breakdown, and PARP cleavage were observed, indicating apoptosis (3–6).

ES-285 inhibited the growth of human tumor xenografts in mice with significant tumor growth arrest against s.c. implanted PC-3 human prostate [<1% treated versus control (T/C)] and MRI-H-121 human renal (28% T/C) tumor xenografts administered on a q4d × 3, i.p. schedule. In a hollow fiber model, ES-285 showed significant activity against s.c. implanted SK-HEP-1 hepatoma tumor cells at the maximum tolerated dose (MTD; 14% T/C) and 1/2 MTD (19% T/C) administered on a qd × 5 i.v. schedule. Pharmacokinetic studies in rats following bolus i.v. dosing suggested that ES-285 had a high volume of distribution (mean V_{ss} at 1/2 MTD, 96 L/kg; SD, 18 L/kg), with relatively rapid plasma clearance (mean $t_{1/2}$ at 1/2 MTD, 3.4 h; SD, 0.4 h). No clear schedule dependency was identified preclinically. Toxicology studies in the

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mouse, rat, dog, and monkey revealed no myelotoxic effects, but identified myocardial degeneration, hepatic transaminitis, renal tubular necrosis, and injection site phlebitis as potential toxicities (1).

The primary objectives of this study were to determine the MTD, recommended phase II dose, and toxicities associated with ES-285 administered as a 24-hour i.v. infusion every 3 weeks to patients with advanced solid tumors. This was one of a range of practically feasible schedules explored by the sponsor. Three other schedules were studied in separate phase I trials: a 3-hour i.v. infusion given daily for 5 days every 3 weeks; a 3-hour i.v. infusion administered weekly on a 3 week cycle; and a 3-hour i.v. infusion given once every 3 weeks (7). Secondary objectives were to determine preliminary information on the pharmacokinetics and antitumor activity of ES-285 on this schedule. Finally, gene expression profiling of patient tissue samples was done pre- and post-treatment to identify potential mechanistic and pharmacodynamic markers of drug action *in vivo*.

Patients and Methods

This was a two-center collaborative study by the Royal Marsden Hospital (Sutton, United Kingdom) and the Erasmus University Medical Centre (Rotterdam, the Netherlands). The study was approved by the Ethics Committees of the participating institutions.

Eligibility Criteria

Patients with a histologically or cytologically confirmed malignant solid tumor were eligible for the study if no standard therapy would reasonably have been expected to result in cure or palliation. Other inclusion criteria included age of 18 to 70 y; life expectancy >12 wk; Eastern Cooperative Oncology Group performance status ≤2; and adequate cardiac, liver, renal, and bone marrow function as defined by left ventricular ejection fraction ≥50%; aspartate aminotransferase and alanine aminotransferase ≤2.5× and bilirubin ≤1.5× the upper limit of institutional normal; serum creatinine <1.5 mg/dL or calculated creatinine clearance >60 mL/min; hemoglobin ≥9g/dL; neutrophils ≥1.5 × 10^9/L; and platelets ≥100× 10^9/L. Women and men of reproductive potential had to use a medically acceptable method of contraception throughout the treatment period and for 3 mo after discontinuation of treatment. Patients were excluded from the study if they had symptomatic or progressive brain metastases; were immunocompromised; had an active infection or intercurrent illness; or had received chemotherapy, radiotherapy, or biological therapy within 4 wk before administration of ES-285. Patients with increased cardiac risk were excluded if there was a history of unstable angina, myocardial infarction, congestive heart failure, valvular heart disease, clinically significant arrhythmia, untreated hyperlipidemia, or uncontrollable hypertension.

Drug Administration

ES-285 was provided as a lyophilized powder in glass vials (PharmaMar) containing 50 mg of ES-285 (8). The drug product was reconstituted by adding 5 mL of water, and further diluted with 5% dextrose to a final volume of 500 mL for injection. The resulting solution was infused over 24 h through a peripheral or central i.v. line (9). Drug administration was repeated every 3 wk provided any toxicity the patient experienced had recovered to grade 1 or resolved completely.

Dosage and Dose Escalation

A dose of 4 mg/m^2 (equivalent to 1/30th of the MTD in mice) was considered a safe starting dose in humans, based on preclinical toxicology studies. Patients were assigned sequentially to escalating dose level cohorts of at least three patients. No within-patient dose escalations were permitted. At the start of a new dose level, the first patient treated was observed for 21 d before subsequent patients could be treated at that dose. The dose of ES-285 was doubled between cohorts until grade 2 drug-related toxicity was seen. Dose-limiting toxicity (DLT) was defined as a neutrophil count <0.5 × 10^9/L for longer than 5 d; neutrophils <1.0 × 10^9/L accompanied by a fever (≥38°C); platelets <25 × 10^9/L; decrease in the left ventricular ejection fraction ≥20% from baseline; any other grade 3 to 4 nonhematologic toxicity except nausea and vomiting responding to antiemetics; or toxicity leading to a delay in starting the next cycle of treatment exceeding 2 wk. If 1 of 3 patients experienced DLT by day 21 at any given dose level, a further 3 patients were to be enrolled at that level. The dose would only be further escalated if DLT were limited to one patient. If ≥2 of 6 patients experienced DLT at any given dose level, that level would be considered the MTD. The recommended dose for phase II studies was defined as the highest dose level at which <2 of 6 patients experienced DLT.

Study Assessments

Pretreatment and on-study assessments included medical history, physical examination, Eastern Cooperative Oncology Group performance status, vital signs, weight, full blood count, full biochemistry profile including serum cardiac troponin I and creatine kinase, pregnancy test, electrocardiogram, and multigated acquisition scans. Renal function was monitored by regular measurement of urea and creatinine, calculation of creatinine clearance using the Cockcroft and Gault method, and urinalysis. Toxicity assessments were continued weekly until 28 d after the last treatment. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 2.0. Tumor volume was measured by computed tomography scan at baseline and after every two cycles of treatment, according to the Response Evaluation Criteria in Solid Tumors criteria (10). Serial measurements of serum tumor markers were done in patients with applicable tumor types.

Pharmacokinetic Analysis

Fifteen blood samples of 5 mL were taken during the first cycle of treatment from the arm opposite the site of infusion at the following time points: immediately before the start of infusion; 2 and 12 h after the start of infusion; 10 min before the end of infusion; and 15 min, 30 min, 1, 1.5, 2, 3, 4, 5, 7,

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24, and 48 h after the end of infusion. Additional pharmacokinetic sampling was done at 96 and 120 h after the end of infusion in patients treated at 200 and 256 mg/m^2. Pharmacokinetic samples were evaluated using liquid chromatography with tandem mass spectrometry. The lower limit of quantitation of the assay was 1 ng/mL, with acceptable accuracy and precision at these levels (coefficient of variation <20%; ref. 11).

Pharmacokinetic parameters were calculated for each patient using standard noncompartmental methods. The peak plasma concentration (C_{max}) represented the highest observed value read from the raw data. For each patient, the area under the concentration versus time curve was calculated up to the last detectable concentration (AUC_{0-t}), with the log-linear trapezoidal rule and extrapolated to infinity (AUC_{0-inf}) using the terminal rate constant (k), estimated by linear regression analysis of the final concentration-time data. The apparent terminal elimination half-life ($t_{1/2}$) was calculated as the natural log of 2 divided by k. Total plasma clearance (Cl) was dose divided by AUC_{0-inf}. The volume of distribution at steady state (V_{ss}) was calculated as the mean residence time multiplied by the clearance. The calculations were made using WinNonlin, version 5.0.1 (Pharsight).

Pharmacodynamic Analysis

Pharmacodynamic studies were undertaken using patient tissue samples to assist in the selection of drug dose and schedule for further studies, and to help understand the mechanism of biological action of the drug *in vivo*. Blood samples were taken from all patients, but sequential skin or tumor biopsies were optional. Pharmacodynamic samples were taken immediately before and after the 24-h infusion of ES-285 in cycle 1. Blood samples of 20 mL were drawn into PAXgene blood RNA tubes to quickly lyse cells and stabilize the blood RNA profile before freezing at −80° C. Skin biopsies were obtained using a 5-mm-diameter Acupunch (Acuderm) and snap-frozen in liquid nitrogen. Pharmacodynamic samples were analyzed centrally at the Institute of Cancer Research, Sutton, United Kingdom. Whole blood sample was extracted using the PAXgene blood RNA kit following the manufacturer's instructions (PreAnalytix Gmbh). Frozen skin biopsies were homogenized directly into TRizol denaturing solution (Invitrogen Ltd.), and RNA was isolated using a method similar to that developed by Chomczynski and Sacchi (12). RNA from blood samples and skin biopsies was further purified with the Qiagen RNeasy kit (Qiagen), then amplified using the MessageAmp II aRNA method (Ambion; ref. 13). RNA quality was ensured pre- and post-amplification with the Agilent 2100 Bioanalyser (Agilent Technologies). RNA samples were labeled using the Cyscribe post-labeling kit (Amersham Biosciences UK Ltd.; ref. 14). Cy5-labeled patient total RNA samples were hybridized with Cy3-labeled Universal Human Reference RNA (Stratagene) onto 22k cDNA gene expression microarrays, as previously described (15). After hybridization, slides were washed and scanned with an Axon 4000A scanner and Genepix 4.0 software (Axon Instruments).

Statistics

Evaluation of the safety and efficacy data in this study was primarily descriptive; as inferential methods were not used, statistical power considerations were not relevant for this. All patients who received at least one dose of ES-285 were to be included in the safety evaluation. Analysis of gene expression data was done using Genespring 7.2 (Silicon Genetics). Raw data were LOWESS normalized (16) and filtered to produce quality-controlled gene lists, which excluded unreliable or unchanging genes. Correlations between levels of gene expression and clinical variables were explored using the Welch t test for binary variables and ANOVA for continuous variables, generally using a P value of 0.05. Multiple testing correction was done with either Bonferroni (17) or Benjamini-Hochberg methods (18). Overrepresentation of functional categories within gene lists were identified using expression analysis systematic explorer (EASE) analysis. This identifies functional categories of genes enriched in the gene list of interest, compared with the list of all genes on the array, using a variant of the Fisher exact test (19).

Results

Patient Characteristics and Treatments Administered

Twenty-nine patients were registered for this study between October 2003 and October 2005. One registered patient became unfit for the study before receiving ES-285. Twenty-eight patients were treated with 72 courses of ES-285 across eight dose levels. The median number of courses received was 2 (range, 1–6). Patient characteristics are listed in Table 1.

Dose Escalation, DLTs, and MTD

Cohorts of three to four patients were treated at the following dose levels: 4 mg/m^2 (n = 4), 8 mg/m^2 (n = 4), 16 mg/m^2 (n = 3), 32 mg/m^2 (n = 4), 64 mg/m^2 (n = 3), 128 mg/m^2 (n = 3), 256 mg/m^2 (n = 4), and 200 mg/m^2 (n = 3). The dose was doubled between cohorts until at least grade 2 drug-related toxicity was seen. This did not occur until the 256 mg/m^2 cohort, when two of four patients experienced DLT, thus defining MTD. Both patients experienced a grade 1 to 2 fever following drug administration, then a reversible grade 3 increase in transaminases (aspartate aminotransferase and alanine aminotransferase). Patient 24, a 28-year-old woman with metastatic chondrosarcoma, had no liver metastases, with normal liver function at baseline. Transaminases were elevated on day 3, peaked on day 5, and had normalized by day 15. Following her DLT in cycle 1, she went on to receive a further five cycles at a reduced dose of 128 mg/m^2, with no further transaminitis. Patient 26, a 48-year-old man with colorectal cancer metastatic to liver, also had adequate liver function at baseline. Transaminases were elevated on day 4, peaked on day 6, and returned to grade 1 by day 15. In addition to grade 3 transaminitis, he also experienced dose-limiting neurologic toxicity with grade 3 tremor, ataxia, hazy vision, and confusion on day 4, lasting less than 24 hours. He recovered without sequelae. These neurologic symptoms were also considered drug related and a DLT. He received no further treatment with ES-285.

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The next cohort of patients was treated at a reduced dose of 200 mg/m^2. However, of three patients treated at this dose level, patient 29 died following drug-related central neurologic toxicity. This patient had colorectal cancer with liver metastases and had previously been treated with two chemotherapy regimens before receiving ES-285. On day 1, he developed a grade 2 injection site reaction and grade 2 fever, which was treated with paracetamol and oral co-amoxyclav. On day 3, he became confused and incontinent of urine, and by day 4 had become unconscious. The patient was treated with i.v. meropenem and aciclovir empirically, although analysis of his cerebrospinal fluid revealed no evidence of infection by microscopy, culture, and viral PCR. Cranial magnetic resonance imaging was also normal. The patient subsequently developed multiorgan failure, which was refractory to therapy, and died 6 days after ES-285 administration. A postmortem examination revealed no focal cerebral lesions to explain the neurologic symptoms and signs, which are presumed to have been caused by ES-285.

Other Toxicities

Non-DLTs included injection site reaction, nausea, fatigue, and fever (see Table 2). Injection site reactions were seen in half the patients in the 8 mg/m^2 cohort and were partially alleviated in subsequent cohorts with the use of central venous catheters. Seven patients reported mild to moderate nausea, one with associated vomiting. One patient with renal cell cancer and a prior nephrectomy came off study after 16 weeks of treatment with ES-285 at 8 mg/m^2 due to progressive renal impairment, which recovered after he was taken off study. Two patients had an asymptomatic (Common Toxicity Criteria for Adverse Events grade 2) drop in left ventricular ejection fraction. One patient treated at 32 mg/m^2 had left ventricular ejection fraction of 57%, which after cycle 1 was 45% and after cycle 2 was 56%. The other patient was treated at 128 mg/m^2 and corresponding values were 68%, 62%, and 51%.

Pharmacokinetics

Pharmacokinetic studies were done in all 28 of the evaluable patients during the first course of ES-285. A summary of these data is given in Table 3. Both C_{max} and AUC seemed to increase dose-proportionally (Fig. 1). ES-285 has a long elimination half life (mean, 22 hours; SD, 13 hours) and a large volume of distribution (mean, 1793 L/m^2; SD, 1017 L/m^2). Plasma protein binding was estimated at 99.75%. The plasma concentration-time profiles of ES-285 were generally characterized by peak concentrations achieved during the second half of the 24-hour infusion. The three patients experiencing DLT had the highest C_{max} and AUC values (Fig. 1).

Antitumor Activity

No objective tumor responses were seen, but stable disease was recorded in nine patients. This lasted for six cycles (18 weeks) in two patients: one with metastatic renal cell carcinoma in the 8 mg/m^2 cohort and the other with metastatic prostate carcinoma in the 200 mg/m^2 cohort. There were no significant reductions in tumor markers.

Pharmacodynamic Studies

Pre- and post-treatment whole blood samples and skin biopsies were obtained for all 28 patients except for 2 patients who declined to have skin biopsies taken. Potential drug-induced changes in gene expression were sought by correlating the level of fold change with dose of ES-285 administered. Fold change in gene expression following drug treatment was calculated by dividing the posttreatment value for each gene by its pretreatment value. Correlation by ANOVA of change in gene expression with dose administered identified 909 dose-responsive genes in blood samples ($P < 0.05$). The same analysis in skin biopsies samples identified 903 genes ($P < 0.05$). Gene set enrichment analysis of these 909 (blood) and 903 (skin) gene lists was done using the EASE algorithm. This identifies functional categories of genes enriched in the gene list of interest compared with the list of all genes on the array, using a variant of the Fisher exact test (19). In both surrogate tissues, cell cycle genes are more significantly overrepresented than

Table 1. Patient characteristics

Patients	$n = 28$
Sex	
Male	25
Female	3
Age (y)	
Median (range)	57 (24–78)
ECOG performance status	
0	5
1	21
2	2
Primary tumor	
Colorectal	7
Prostate	5
Renal	3
Pancreas	2
Malignant melanoma	2
Osteosarcoma	2
Chondrosarcoma	2
Soft tissue sarcoma	1
Esophageal	1
Mesothelioma	1
Bladder	1
Adenocarcinoma unknown primary	1
Previous treatment	
Surgery	23
Radiotherapy	15
Systemic therapy	28
Cytotoxic therapy	22
Molecular targeted therapy	7
Hormone therapy	6
Immunotherapy	4
No. of prior systemic regimens	
0	0
1–3	18
≥4	10
No prior therapy	0

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

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Table 2. Dose escalation scheme and treatment-emergent toxicities of ES-285

Dose level (mg/m^2)	No. of patients (cycles)	Injection site reaction		Nausea/ vomiting		Fatigue		Fever		Transaminitis		Central neurotoxicity	
		Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
4	4 (6)	—	—	2 (2)	—	—	—	—	—	—	—	—	—
8	4 (13)	2 (5)	—	1 (5)	—	—	—	—	—	—	—	—	—
16	3 (10)	—	—	1 (2)	—	—	—	—	—	—	—	—	—
32	4 (9)	2 (2)	—	—	—	—	—	—	—	—	—	—	—
64	3 (8)	2 (3)	—	1 (2)	—	—	—	—	—	—	—	—	—
128	3 (5)	—	—	—	—	3 (5)	—	—	—	—	—	1 (1)	—
256	4 (12)	1 (2)	—	1 (2)	1 (1)	—	—	3 (4)	—	—	2 (2)*	—	1 (1)*
200	3 (9)	1 (1)	—	1 (2)	—	—	—	—	—	1 (1)	—	—	1 (1)*

NOTE: The number of patients experiencing toxicity during cycle 1 is shown for each dose level. Total episodes of toxicity during all cycles of treatment are shown in parentheses.
*Dose-limiting toxicities.

any other functional group (Table 4A, with full gene lists in Supplementary Table S1).[4] This overall apparent functional consistency is seen despite an overlap of <10% between individual genes in these two gene sets. The 69-gene overlap between blood samples and skin biopsies potentially represents a core gene expression signature for the pharmacodynamic effects of ES-285 in surrogate tissues of blood and skin (Supplementary Table S2).[4] In a separate analysis, a subset of all dose-responsive changes in gene expression following treatment were not seen until a dose of at least 128 mg/m^2. These included genes involved in cell cycling, cell adhesion, and the actin cytoskeleton (Table 4B, with full gene lists in Supplementary Table S3).[4]

Discussion

ES-285, a novel agent isolated from the marine mollusc *M. polynyma*, exhibits nanomolar antitumor activity against a broad range of human cancer cell lines *in vitro* with *in vivo* preclinical activity. Although its precise mechanism of antitumor action is not yet fully understood, *in vitro* studies have revealed that treatment with this agent is associated with modulation of RHO protein and ceramide signaling, followed by disassembly of actin stress fibers, G_2-M cell cycle arrest, and apoptosis (4–6). *In vivo* studies showed that ES-285 inhibited the growth of a range of human tumor xenografts, with particular activity noted against hepatoma, renal, and prostate cancers (1). Preclinical toxicology studies revealed no myelotoxic effects, but identified myocardial degeneration, hepatic transaminitis, renal tubular necrosis, and injection site phlebitis as potential toxicities (1).

We now report on the clinical evaluation of ES-285 in patients with advanced solid cancers. In this phase I trial, ES-285 was administered as an i.v. infusion over 24 hours every 3 weeks. The starting dose was determined at 4 mg/m^2, equivalent to 1/30th of the MTD in mice. Doses up to and including 128 mg/m^2 were generally well tolerated, with manageable toxicities including injection site reaction, nausea, fatigue, and fever. MTD was determined at 256 mg/m^2, with two of four patients experiencing DLT, as defined in the study protocol. Both patients had a reversible grade 3 increase in transaminases, consistent with the preclinical toxicology. In addition to this, one of these patients experienced reversible grade 3 central neurotoxicity with confusion, hazy vision, tremor, and ataxia lasting up to 24 hours. This was not a toxicity predicted from animal studies (1), yet was considered to be drug related. The next two patients were treated at 200 mg/m^2 without significant toxicity; however, the third patient treated at this dose also developed central toxicity and died 6 days after ES-285 administration. Recruitment to the study was suspended while a postmortem was carried out, and after no other cause of death could be identified, this event was considered drug related. Patients were not recruited to an expanded cohort due to the overall risk-benefit balance seen in the study to date. Thus, we cannot exclude the possibility that 200 mg/m^2 was MTD. However, safety evaluation proceeded with ES-285 using other schedules, including patients with intracerebral malignancies (7). The toxicity profile of ES-285 administered using a 3-hour i.v. infusion 3-weekly is very similar to that reported here. DLTs were increased liver transaminases and central neurotoxicity. Other toxicities included phlebitis, fever, and headache. MTD was reported as 256 mg/m^2, and at the time of reporting, the tentative recommended dose was 160 mg/m^2 (7).

In this heavily pretreated population, there were no objective tumor responses noted and no reductions in tumor markers. However, stable disease was recorded in nine patients. This lasted for six cycles (18 weeks) in two patients: one with renal cell carcinoma and the other with prostate carcinoma. These were two of the tumor types noted to be most sensitive to ES-285 based on *in vivo* human tumor xenograft studies (1).

The pharmacokinetic profile of ES-285 administered as a 24-hour i.v. infusion once every 3 weeks was characterized

[4] Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org).

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Table 3. Summary of ES-285 pharmacokinetics (first course)

Dose (mg/m^2)	C_{max} (ng/mL)	AUC (ng h/mL)	$t_{1/2}$ (h)	Cl (L/h)	Cl (L/h/m^2)	V_{ss} (L/m^2)
4 (n = 0)*	—	—	—	—	—	—
8 (n = 1)*	3	84	17	163	96	2,010
16 (n = 1)*	4	99	5	274	161	472
32 (n = 4)	11 (7–18)	199 (134–224)	5 (2–7)	335 (286–455)	169 (143–239)	837 (234–2,009)
64 (n = 3)	27 (17–39)	459 (387–525)	17 (14–20)	289 (256–342)	143 (122–171)	1,270 (1,013–1,428)
128 (n = 3)	69 (49–100)	1,244 (1,056–1,438)	34 (29–37)	187 (160–206)	104 (89–121)	1,908 (1,292–2,433)
200 (n = 3)	91 (86–100)	2,238 (1,963–2,457)	37 (30–48)	198 (158–245)	91 (83–102)	2,753 (2,139–3,527)
256 (n = 4)	106 (91–124)	2,486 (1,189–3,510)	28 (21–32)	257 (109–456)	123 (73–217)	2,611 (1,615–4,051)
Mean (n = 19)	N/A	N/A	22	254	128	1,793

NOTE: Values are expressed as median (range).
Abbreviations: AUC, area under the plasma drug concentration_time curve; Cl, clearance; C_{max}, maximum plasma drug concentration following single infusion; N/A, not applicable; $t_{1/2}$, terminal phase half_life; Vss, volume of distribution at steady state.
*Plasma drug concentrations in 10 patients treated with 4 to 16 mg/m^2 ES_285 were low and frequently undetectable, thus precluding any meaningful non-compartmental evaluation.

by a linear relationship between dose and drug exposure (C_{max} and AUC). Reported *in vitro* studies showed an average growth inhibitory IC_{50} of 80 nmol/L, or 26 ng/mL for cells grown in FCS (3). Average C_{max} values in 128, 200, and 256 mg/m^2 correspond to 2.7×, 3.5×, and 4.1× IC_{50}, respectively; however, ES-285 is highly protein bound *in vivo*. The three patients experiencing DLT had the highest C_{max} and AUC values, suggesting that toxicity from ES-285 on this schedule may be exposure dependent.

Gene expression profiling studies *in vitro* have reported that ES-285 modulates the expression of genes involved in the regulation of the actin cytoskeleton, cell cycle, and apoptosis (3). Pharmacogenomic studies with patient tissue samples were undertaken to help further our understanding of the mechanism of action of ES-285 *in vivo* and to identify potential pharmacodynamic markers of drug action. Paired patient surrogate tissue samples were taken before and after the 24-hour infusion of ES-285 for gene expression profiling by cDNA microarray. To minimize the number of unnecessary tumor biopsies, we elected to seek only accessible surrogate tissue samples (blood samples and optional skin biopsies) in the initial stages of the trial. Tumor biopsies were planned once a biologically active dose range was reached. However, because the trial was concluded early due to toxicity, none were obtained.

The incorporation of "omic" technologies into clinical drug development is an area both novel and challenging (20). Whereas many are convinced that biomarkers will help deliver better "personalized" therapy (21–24), the optimal use of biomarkers in phase I clinical trials is an area of some

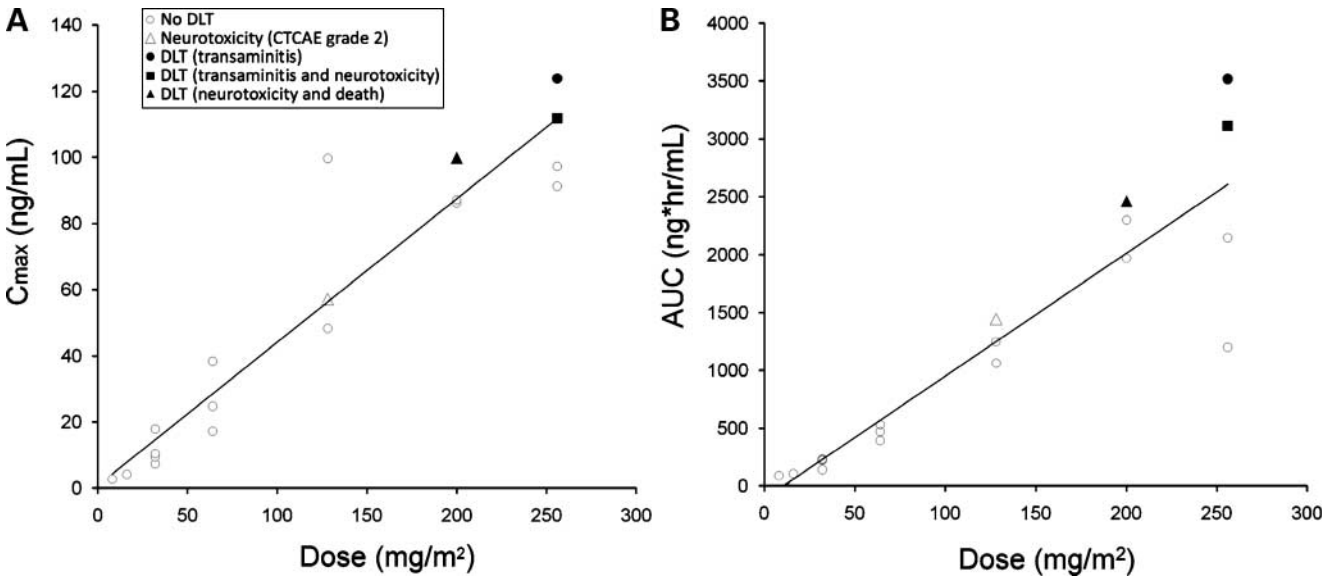

Figure 1. ES-285 pharmacokinetics. Relationship between dose and C_{max} (**A**) and between dose and AUC (**B**). Patients experiencing DLT are shown according to the legend.

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Table 4. Functional categories most significantly overrepresented among genes whose changed expression level following treatment with ES-285 correlates with increasing dose

	Blood samples		Skin biopsies	
	Functional category	EASE score	Functional category	EASE score
(A) All dose levels	Cell proliferation	0.00003	Cell cycle	0.001
	Cell cycle	0.0001	Mitotic cell cycle	0.003
	G_2-M transition of mitotic cell cycle	0.005	Cell proliferation	0.009
	Protein catabolism	0.01	Nuclear division	0.02
	S phase of mitotic cell cycle	0.01	Biological process unknown	0.03
	G_1-S transition of mitotic cell cycle	0.01	M phase	0.03
	Antiapoptosis	0.02	Telomere maintenance	0.04
	Negative regulation of cell cycle	0.02	Coenzyme and prosthetic group metabolism	0.04
	Mitotic cell cycle	0.02	S phase of mitotic cell cycle	0.04
	DNA replication	0.02	Mitosis	0.05
	Regulation of CDK activity	0.03	Amino acid biosynthesis	0.05
	Ubiquitin cycle	0.03	M phase of mitotic cell cycle	0.05
(B) 128 mg/m^2 or higher	Cell proliferation	0.02	Actin filament	0.01
	Cell cycle	0.04	Amino acid metabolism	0.02
	Obsolete cellular component	0.04	Calmodulin binding	0.02
	Cytoplasm	0.06	Amino acid and derivative metabolism	0.02
	Cytoskeletal protein binding	0.08	Enzyme inhibitor activity	0.03
	Sulfotransferase activity	0.08	Actin cytoskeleton	0.03
	Transferase activity	0.09	Nitrogen compound metabolism	0.03
	Amine metabolism	0.10	Amine metabolism	0.03
	Obsolete molecular function	0.10	Cell adhesion	0.03
	Cell growth and/or maintenance	0.11	Phosphate transport	0.04
	Transferase activity	0.12	Collagen	0.04
	Protein phosphatase regulator activity	0.13	Cell adhesion molecule binding	0.05
	Phosphatase regulator activity	0.13	Cadherin binding	0.05
	Cytoskeleton	0.13	Enzyme regulator activity	0.05
	Cell organization and biogenesis	0.14	Structural molecule activity	0.07
	Physiologic process	0.17	Inorganic anion transport	0.07
	Regulation of cell cycle	0.18	Calcium ion binding	0.07
	Regulation of cell proliferation	0.18	Promyelocytic leukemia body	0.07
	Actin binding	0.19	Anion transport	0.07

NOTE: (A) All dose levels. (B) 128 mg/m^2 or higher. Functional categories are ranked by the degree of their overrepresentation based on a variant of the one-tailed Fisher exact probability (EASE score). Categories most highly overrepresented are at the top. A (*top row*): all dose levels. Correlation of change in gene expression with dose administered by ANOVA ($P < 0.05$). Full gene lists are shown in Supplementary Table S1.[4] B (*bottom row*): 128 mg/m^2 or higher. The list of all genes from blood samples was filtered to only include genes with <1.5-fold change in expression in all five lower dose levels (4–64 mg/m^2) and >1.5-fold change in expression in at least two of three higher dose levels (128, 200, and 256 mg/m^2). The list of all genes from skin biopsies was filtered to include genes with <1.5-fold change in expression in three of five lower dose levels and >1.5-fold change in expression in at least two of three higher dose levels. Full gene lists are shown in Supplementary Table S3.[4]

Abbreviation: CDK, cyclin-dependent kinase.

debate (25, 26). In the present study, dose-responsive changes in gene expression were identified in surrogate tissues following treatment with ES-285. Cell cycle genes were the most significantly overrepresented functional group among dose-responsive genes in both blood samples and skin biopsies, consistent with cell cycle effects observed *in vitro* (3). Mechanistically relevant changes in gene expression were seen at doses of 128 mg/m^2 or higher, including genes involved in cell cycling, cell adhesion, and the actin cytoskeleton. These observations suggest that 128 mg/m^2 may potentially be a biologically active dose of ES-285 administered on this schedule, despite the lack of clinical toxicity seen in the three patients treated at this dose level.

In conclusion, ES-285 showed an acceptable safety profile at doses up to 128 mg/m^2. At this dose level, pharmacokinetic data indicated that pharmacologically relevant concentrations had been achieved, and pharmacogenomic studies indicated consistent, dose-responsive changes in expression of genes of potential biological relevance. This study identified transaminitis and neurotoxicity as the DLTs associated with a 24-hour infusion of ES-285 given once every 3 weeks. One patient died following drug-related neurotoxicity. Nine patients recorded stable disease as their best response in a range of tumor types. Gene expression profiling of surrogate tissues yielded information that could potentially assist further drug development and patient selection. We propose that this approach merits more widespread utilization, particularly in the clinical evaluation of novel agents whose precise molecular mechanism of action is unclear.

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Disclosure of Potential Conflicts of Interest

L. Lopez-Lazaro and B. de las Heras: employees, PharmaMar. I. Judson and J. Verweij: advisory board honoraria, PharmaMar. S. Kaye: Board of Directors, PharmaMar. No other potential conflicts of interest were disclosed.

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