Phase I Clinical and Pharmacokinetic Study of Kahalalide F in Patients with Advanced Androgen Refractory Prostate Cancer

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ABSTRACT

Purpose: **The purpose is to determine the maximum tolerated dose, profile of adverse events, and dose-limiting toxicity of Kahalalide F (KF) in patients with androgen refractory prostate cancer. Furthermore, the pharmacokinetics after KF administration and preliminary antitumor activity were evaluated. KF is a dehydroaminobutyric acid–containing peptide isolated from the marine herbivorous mollusk, *Elysia rufescens*.**

Experimental Design: **Adult patients with advanced or metastatic androgen refractory prostate cancer received KF as an i.v. infusion over 1 hour, during five consecutive days every 3 weeks. The starting dose was 20 μg per m^2 per day. Clinical pharmacokinetics studies were done in all patients using noncompartmental analysis. Prostate-specific antigen levels were evaluated as a surrogate marker for activity against prostate cancer.**

Results: **Thirty-two patients were treated at nine dose levels (20-930 μg per m^2 per day). The maximum tolerated dose on this schedule was 930 μg per m^2 per day. The dose-limiting toxicity was reversible and asymptomatic Common Toxicity Criteria grade 3 and 4 increases in transaminases. The recommended dose for phase II studies is 560 μg per m^2 per day. Pharmacokinetics analysis revealed dose linearity up to the recommended dose. Thereafter, a more than proportional increase was observed. Elimination was rapid with a mean (SD) terminal half-life ($t_{1/2}$) of 0.47 hour (0.11 hour). One patient at dose level 80 μg per m^2 per day had a partial response with a prostate-specific antigen decline by at least 50% for ≥4 weeks. Five patients showed stable disease.**

Conclusions: **KF can be given safely as a 1-hour i.v. infusion during five consecutive days at a dose of 560 μg per m^2 per day once every 3 weeks.**

INTRODUCTION

Kahalalide F (KF) is one of a family of dehydroaminobutyric acid–containing peptides isolated from the herbivorous marine species of mollusk, *Elysia rufescens*, an organism living in the seas near Hawaii (1, 2). The green algae *Bryopsis* sp. also produces some of Kahalalide peptides in smaller yields (1, 2). The structure of KF (Fig. 1) contains a lateral chain and a cycled region with the molecular formula $C_{75}H_{124}N_{14}O_{16}$. KF is the largest and most active of the seven natural compounds isolated from *E. rufescens*. Six cyclic (peptides A-F) and one acyclic analogue (peptide G) are known (3).

KF displays both *in vitro* and *in vivo* antitumor activity in various solid tumor models, including colon, breast, non–small cell lung, and in particular prostate cancer. *In vitro* antiproliferative studies showed activity among certain prostate cancer cell lines (PC-3, DU-145, T-10, DHM, and RB), but no activity was found against the hormone-sensitive LnCAP (4). *In vivo* models also confirmed selectivity and sensitivity of the prostate tumor xenograft derived from hormone-independent prostate cancer cell lines, PC-3 and DU-145 (4). Further *in vitro* evaluation showed that this activity is selective but not restricted to prostate tumor cells (4).

Hormone therapy (i.e., androgen ablation) is the first therapy of choice in advanced prostate cancer with effectiveness in reducing tumor growth, but the median duration of response is not longer than ~15 months (5, 6). Radiotherapy and chemotherapy are used to control symptoms. Despite many years of efforts to develop effective chemotherapy, few effective drugs have been identified (7, 8). Recently, two phase III trials have shown a survival advantage of docetaxel-based regimens in the treatment of patients with advanced, androgen-independent prostate cancer. One (Southwest Oncology Group 99-16) reported a 23% improvement in survival in men treated with docetaxel and estramustine compared with the current standard, mitoxantrone plus a glucocorticoid (7). The other showed an improved overall survival and increased prostate-specific antigen (PSA) and pain responses of an every-3-week regimen with docetaxel plus prednisone versus a similar control group (mitoxantrone plus prednisone; ref. 8). Consequently, a docetaxel-containing regimen has become a standard for the first-line therapy of patients with advanced androgen-independent prostate carcinoma.

Therefore, a phase I study with KF has been specifically designed for treatment of patients with advanced or metastatic androgen-refractory prostate cancer.

Received 8/20/04; revised 10/18/04; accepted 12/13/04.
Grant support: Pharma Mar S.A. Sociedad Anónima Unipersonal (Madrid, Spain).
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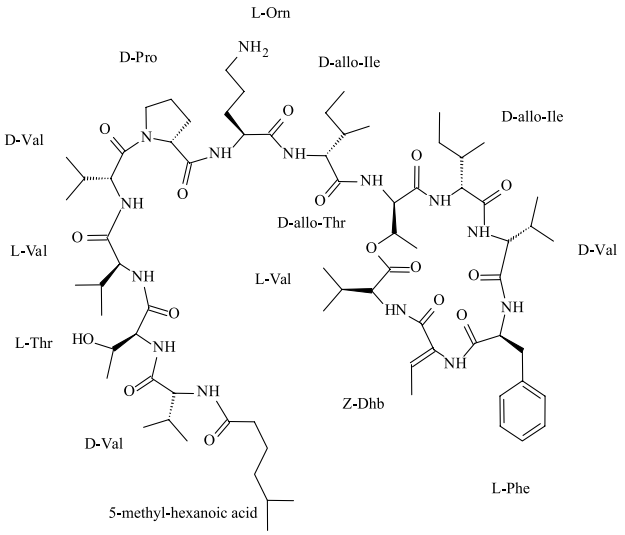

Fig. 1 The structural formula of Kahalalide F. The fatty acid group is connected to the lateral side. Residues of Thr-Ile-Orn-Pro-Val-Val-Thr-Val and 5-methylbenzoic acid form the lateral chain. The cycled region contains Phe-Val-Ile-Thr-Val and dehydrobutyric acid.

The primary mechanism of action of KF has not been identified yet, although multiple targets have been found and each is a membrane-associated event that may be related to the hydrophobic nature of the compound. COMPARE analysis was negative (9) suggesting that KF has a novel mechanism of action. A cell cycle block in G_0-G_1 has been determined in a variety of tumor cell lines that include prostate (DU145), cervical (HeLa), colon (HT29), and head and neck (HN30). Earlier *in vitro* studies identified some mechanisms of action. Cells exposed to biologically relevant concentrations of KF detach from their substrate and become markedly swollen associated with the formation of large intracellular vacuoles (10). The earliest studies have identified lysosomes as the most likely intracellular target based upon the evidence that KF disrupts the integrity of lysosomal membranes (10, 11). If the mechanism of action is at the lysosomal level, then those tumor cells containing a higher proportion of lysosomes would be more suitably treated with KF. This is a feature of prostate cells in which lysosomes are actively secreting proteins, acid, and alkaline phosphatases and contain high concentrations on zinc (12–15). It has been recently published that tumor cells with higher HER-2/*neu* and/or HER3 expression are particularly sensitive to KF, which induces a form of cell death that is independent of caspase, cathepsin B, or cathepsin D activity, and correlates with down-regulation of Akt signaling (16).

Preclinical toxicity studies of KF using single- and multiple-dose (daily for 5 days) schedules were done in male and female rats (17). The maximum tolerated dose (MTD) was estimated to be 300 μg/kg (i.e., 1,800 μg/m^2) using single-dose schedules. Single-dose administration of KF at 150 and 300 μg/kg produced nephrotoxicity, neurotoxicity, and injury to blood vessels and surrounding tissue at the injection site. When KF was given once daily for five consecutive days at a dose of 80 μg/kg per day (400 μg/kg total dose), slightly decreased body weight gain was the primary drug-related effect. These findings show that fractionation of a lethal or MTD dose of KF by daily administration for 5 days reduced drug-induced toxicity, and seemed to be a viable option for the clinical evaluation of KF (17). Based on the MTD values defined in mice, a starting dose of 20 μg per m^2 per day was selected for this phase I trial, which was equivalent to a total dose of 100 μg/m^2. The marine derived anticancer drugs, including KF, are known for their potencies (18). Therefore, these drugs are given at very low doses.

A phase I dose-escalating study of KF was done in the Netherlands Cancer Institute. The primary objective of this study was to determine the MTD of KF. The secondary objectives were (a) to determine an optimal dose for phase II evaluation, (b) to determine the toxicity profile and pharmacokinetics of KF and the correlation between them, and (c) to document any possible antitumor activity.

MATERIALS AND METHODS

Patient Eligibility Criteria. Patients were eligible if they had histologically or cytologically proven advanced or metastatic androgen-refractory prostate carcinoma. The patients had progressive disease, which was defined as (a) appearance of a new lesion or increase in size (25%) of a measurable lesion, (b) two consecutive increases in PSA documented over a previous reference value, or (c) PSA-negative patients with increasing tumor-related bone pain or needs for analgesia.

Patients who had a failed surgical or medical castration or failure of treatment with leutinizing hormone releasing hormone agonists were included in the study. Antiandrogen therapy had to be discontinued for at least 4 weeks before entry into the study. Previous treatment with any other anticancer pharmacologic therapy should have been discontinued for >4 weeks. Palliative radiotherapy should have ended at least 4 weeks before study entry, and for isotope therapy this period should be at least 6 weeks. Other eligibility criteria included a WHO performance status of 0 to 2 and anticipated life expectancy of at least 3 months. All patients had to have acceptable bone marrow, liver, and renal functions. Serum bilirubin had to be within 2.5 times the normal upper limit, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 times the normal upper limit, and serum creatinine had to be ≤1.5 times the normal upper limit. Patients had to be able and willing to undergo blood sampling for pharmacokinetics. Patients were excluded if they had a history of another malignancy (except cured nonmelanoma skin cancer) or a history of myocardial infarction, uncontrolled angina pectoris, or arrhythmia. Patients were also excluded if they had clinical signs of brain and/or leptomeningeal evidence of tumor, active bacterial infections, or significant liver disease. Further exclusion criteria were preexisting motor or sensory neurotoxicity [Common Toxicity Criteria (CTC) grade ≥2], or hypersensitivity reactions to any drug formulated in Cremophor. The Medical Ethics Committee of our Institute approved the study protocol, and all patients had to give written informed consent.

Treatment Plan and Study Design. KF was given as an i.v. infusion over 1 hour, during five consecutive days every 3 weeks. A starting dose of 20 μg per m^2 per day was selected, which is equivalent to a total dose of 100 μg/m^2. Doses were escalated in decreasing rates and depended on the clinical judgement of the investigators and upon assessment of the safety

profile of the patients. Dose escalation was scheduled as follows: 20, 40, 80, 160, 320, 560, 700, and 930 μg per m^2 per day. At nontoxic dose levels, doses were escalated by 100% and one patient per dose level was treated. After the occurrence of grade 2 toxicity (except asthenia, alopecia, anemia, or nausea and vomiting) the dose level was expanded to three patients with only 50% increase in dosage. If grade 3 or grade 4 toxicity was reached, which were not classified as dose-limiting toxicity (DLT), doses were escalated by 25% steps. Three patients per dose level were then treated. When the MTD was established, six patients were treated at the next lower dose level and sufficient number of patients (at least 12) were included at the recommended dose. The MTD was defined as the dose at which at least two of three or more than three patients experienced DLT. Given that this is a phase I trial and that the primary mechanism of action of KF has not been identified yet treatment duration of at least 6 weeks without any decline in PSA was allowed, to increase the knowledge on this new experimental drug.

Drug Product. KF was supplied as a sterile lyophilized product. Two different sizes of vials containing 50 and 150 μg of KF, respectively, were provided by Pharma Mar (Madrid, Spain). Drug vials were stored at 2°C to 8°C and were protected from light. The reconstitution solution was supplied in ampoules containing 3 mL of 5%/5%/90% v/v/v of Cremophor/ethanol/water. This solution was stable in the original container for at least 24 hours at room temperature and ambient light. The 50-μg vials were reconstituted by adding 1 mL of reconstitution solution. The 150-μg vials were reconstituted by adding 3 mL of reconstitution solution. The drug concentration of the reconstituted drug solution was therefore 49 μg/mL. The resultant solution was colorless, clear, and essentially free of particulate matter. To prepare the drug for i.v. infusion, the reconstituted solution was further diluted in normal saline. KF was given using an infusion set consisting of glass container and silicon tubing. Infusion solutions stored in glass infusion containers at either room temperature or refrigerated conditions, in the dark, were stable for at least 5 days after preparation. The pharmaceutical development has been described before (19).

Patient Evaluation. A complete medical history and physical examination were completed before registration. Before each cycle, the physical examination was repeated and medical history was also obtained within 72 hours of treatment on day 1 of each cycle. Laboratory safety tests were assessed within 1 week previous to treatment. Hematology, coagulation tests, biochemistry, and urinalysis were done within 72 hours previous to administration of the drug in each cycle and thereafter they were checked weekly. On day 2 of each cycle, the liver functions were assessed (bilirubin, alkaline phosphatase, AST, ALT, and γ-glutamyl transferase). Tumor evaluations were done by CT scan, chest X-ray, magnetic resonance imaging, and/or ultrasonography and a bone scan was also done. These were assessed within 3 weeks before study entry and every two cycles. When the response criteria were met, the appropriate tests were repeated 4 weeks later to confirm response. PSA determinations were obtained within 1 week of treatment and every other cycle, as recommended in the 1998 Ontario PSA Clinical Guidelines for follow-up of treatment (20). A PSA decline by at least 50% for ≥4 weeks was considered a response to treatment (21). If PSA values were not decreased after 6 weeks, PSA progression was defined as a ≥25% increase over the baseline value and an absolute increase of at least 5 ng/mL had to be confirmed by a second value at least 2 weeks later (22). In patients whose PSA had decreased but did not meet the PSA response criteria, PSA progression was defined as a ≥25% increase over the baseline value, provided that the absolute increase was of at least 5 ng/mL (22). If patients met the criteria for a PSA response, PSA progression was defined as a ≥50% increase over the baseline value (22). In addition, the PSA doubling time (PSADT) was calculated for all patients as a novel surrogate end point in hormone-refractory prostate cancer. The PSADT was calculated according to the following formula: $PSADT = [\log (2) \times t] / [\log (\text{final PSA}) - \log (\text{initial PSA})]$, where t is the time from initial to final PSA determination.

Although assessment of the antitumor activity was not a primary objective of this study, patients with measurable disease were evaluated according to the RECIST criteria (23). All toxicities were graded according to the National Cancer Institute CTC (24). DLT was defined as grade 4 thrombocytopenia, grade 4 neutropenia lasting longer than 5 days or complicated by fever or infection, any other grade 3 or 4 nonhematologic toxicity (with the exception of untreated nausea, emesis, and hypersensitivity reactions) or grade 3 or 4 increases in transaminases. These toxicities were only considered DLT if they happened during the first treatment cycle.

Pharmacokinetic Studies. Clinical pharmacokinetics studies were done in all patients during the first course of treatment. Blood samples were obtained from patients at 25 time points: preinfusion, 30 minutes after start of infusion; at the end of the infusion, at 15, 30 minutes and 1, 2, 4, 10, and 24 hours after the end of the infusion on days 1 and 5. Blood samples were also obtained before and after infusion on days 2, 3, and 4. The pharmacokinetics sampling schedule was adapted, because the time points 10 and 24 hours after the end of the infusion on days 1 and 5 and the time points on days 2, 3, and 4 were not informative as no KF was quantifiable, even at the highest dose levels explored. For the new schedule blood samples were taken at preinfusion, 30 minutes after start of the infusion; at the end of the infusion, at 15, 30 minutes and 1, 2, 3, and 4 hours postinfusion on days 1 and 5 of course 1. Furthermore, these sampling time points were also used on day 1 of course 2, which allowed us to elucidate whether the pharmacokinetics variables vary from cycle 1 to cycle 2.

Samples of 5 mL each were collected in heparinized tubes from the arm contralateral to that receiving the drug infusion. The blood samples were immediately centrifuged at 4°C at 1,200 × g for 10 minutes. The resulting plasma layer was immediately separated and stored at −20°C until analysis.

Pharmacokinetics studies in urine were done only in patients treated from the moment the MTD was defined. The patient was asked to void just before the start of the infusion. Thereafter, the total urine output was collected at 0 to 24 and 24 to 48 hours. The urine collected during each time interval was thoroughly mixed, the total volume was recorded, and one 8 mL aliquot was stored at −20°C until analysis. The quantitative determination of KF in human plasma and urine was done using reversed-phase high-performance liquid chromatography under basic conditions coupled with positive turboionspray tandem mass spectrometry (liquid chromatography tandem mass spectrometry; ref. 25).

Pharmacokinetic Analysis. Pharmacokinetic analyses of the individual plasma concentration data sets for KF were done using standard model-independent (noncompartmental) methods (WinNonLin Professional 4.0, Pharsight Co., Mountain View, CA). The pharmacokinetic variables included area under the plasma concentration-time curve extrapolated to infinity (AUC) calculated using the linear-log trapezoidal rule and maximum observed plasma concentration (C_{max}). Total body clearance (Cl_{tot}) was calculated as dose/AUC. Volume of distribution at steady state (V_{ss}) was calculated as the product of clearance by mean residence time, that in turn was calculated as the ratio between the area under the first moment of concentration time curve and the AUC minus the actual infusion time divided by 2. The terminal half-life was determined using concentration data in the terminal log-linear phase. All computations used the actual sampling times. The pharmacokinetics variables were reported as mean (SD). Dose linearity was graphically evaluated.

Drug accumulation from day 1 to day 5 of cycle 1 was evaluated by paired *t* test on logarithm transformed values of AUC with calculation of the associated 95% confidence intervals for the difference and back transformation to obtain the ratio of the geometric means.

RESULTS

Patient Characteristics. A total of 32 patients were included in the study. Patient characteristics are presented in Table 1. Median age of the patients was 68 years (range, 49-81) and most patients were in good general condition. All patients had advanced androgen refractory prostate carcinoma and had received prior hormone therapy, radiotherapy, and/or surgery. The patients had metastases to the bone, lymph nodes, liver, and/or lungs.

A total of 106 courses (range, 1-10) of KF were given. The number of patients treated at each dose level and the number of courses given are summarized in Table 2. All patients received at least two courses of KF. Except for one patient at dose level 40 μg per m^2 per day with an WHO performance status 2, who went off study after one course because of brain metastases, and one patient at dose level 560 μg per m^2 per day went off study after the first course because of an emergent spinal cord lesion.

Adverse Events. All patients who had received KF were evaluable for toxicity. One patient per dose level was treated with KF at 20, 40, 80, and 160 μg per m^2 per day. No serious drug-related toxicities were observed at these dose levels. Four patients were treated at 320 μg per m^2 per day, because of rapidly reversible CTC grade 2 aspartate aminotransferase increase, which occurred in the first three patients at this dose level. Therefore, the dose was escalated 33% to 425 μg per m^2 per day. The next dose levels were 560 μg per m^2 per day (+33%), 700 μg per m^2 per day (+25%) and 930 μg per m^2 per day (+33%; Table 2). On day 3 of the first course, one patient at dose level 700 μg per m^2 per day had a dose reduction to 560 μg per m^2 per day due to transaminase elevation. Rapidly reversible CTC grade 3 and 4 transaminase elevation was observed at dose level 560, 700, and 930 μg per m^2 per day. At dose level 930 μg per m^2 per day, this drug-related toxicity was considered dose limiting, as all three included patients had a transient grade 3 or 4 transaminase elevation. Therefore, this dose level was considered to be the MTD. At the next lower dose level, 700 μg per m^2 per day, rapidly reversible CTC grade 3 and 4 transaminase elevations also occurred and therefore the dose was lowered again to 560 μg per m^2 per day on this schedule. The elevation in AST and ALT occurred shortly after start of the KF administration and was rapidly reversible within 3 days to CTC grade 1 and noncumulative. Therefore, the recommended dose (RD) for further testing of KF was determined to be 560 μg per m^2 per day on this schedule.

Table 1 Patient characteristics

	No. patients	% Patients
Tumor type		
Advanced androgen refractory prostate carcinoma		
Total *N*	32	
Median age (range)	68 (49-81)	
Eastern Cooperative Oncology Group performance status		
0	12	38
1	16	50
2	4	12
Previous therapy		
Surgery and hormone therapy	9	28
Surgery, radiotherapy and hormone therapy	23	72
Metastases		
Bone	31	
Lymph nodes	7	
Liver	4	
Lungs	1	

The main treatment-related hematologic adverse events per patient as a function of dose are presented in Table 3. Overall, hematologic toxicity was negligible, and none of the hematologic adverse events were clearly dose related. CTC grade 1 anemia occurred in 22 patients (69%), thrombocytopenia in eight patients (25%), and neutropenia occurred in three patients (9%). CTC grade 2 anemia occurred in eight patients (25%) and neutropenia in two patients (6%) and there were no grade 3 or 4 hematologic adverse events. Biochemical abnormalities per patient as a function of dose are presented in Table 4. CTC grade 2 creatinine increase occurred in two patients (6%) at dose level 560 and 700 μg per m^2 per day. CTC grade 3 bilirubin increase occurred in one patient (3%) at 700 μg per m^2 per day. Transaminase elevations were observed throughout most dose levels reaching CTC grade 3 and 4 at dose levels 560, 700, and 930 μg per m^2 per day.

The main other treatment-related nonhematologic adverse events per patient as a function of dose are presented in Table 5. Five patients (16%) suffered a grade 3 adverse event of any type and there were no grade 4 adverse events. Other toxicities encountered were nausea and hypersensitivity reactions, which usually occurred at the higher dose levels. The symptoms of the hypersensitivity reactions were fever, transpiration, and rubor. Most patients complained about "puncture prick or pruritus" on the palm of the hands shortly after KF infusion.

Pharmacokinetics. Blood samples for the measurement of KF were obtained from all 32 subjects during the first course of treatment. In the last 10 patients blood samples were also

Table 2 Dose escalation

Dose (μg/m²/d)	20	40	80	160	320	425	560	700	930
n	1	1	1	1	4	3	6	12	3
No. courses	4	1	8	4	14	7	16	37	15

obtained during course 2. Complete plasma concentration-time curves of KF were obtained on days 1 and 5. Figure 2 presents plasma concentration-time curves of KF during course 1 in patients receiving 560, 700, and 930 μg per m^2 per day. For KF the lower limit of quantitation was 1.01 ng/mL. Due to the very low doses that were given in this study and the rapid elimination of KF from the body, most subjects had plasma concentrations of KF above the lower limit of quantitation for only up to 3 to 4 hours after the end of the infusion. At the starting dose of 20 μg per m^2 per day, a C_{max} of 1.8 ng/mL was obtained, whereas at 930 μg per m^2 per day the mean C_{max} was 184 ng/mL after the first infusion. Relevant pharmacokinetics variables of KF were calculated and listed in Table 6. A graph of the total KF dose in μg versus C_{max} in ng/mL is presented in Fig. 3. Maximum concentrations of KF were reached at the end of the infusion. After the end of the infusion the drug concentration declined rapidly. A graph of the total KF dose in μg versus the AUC in h ng/mL is presented in Fig. 4 and revealed dose linearity up to the dose of 560 μg per m^2 per day during five consecutive days.

A rapid elimination was observed with a mean terminal half-life of 0.47 hour (0.11 hour). Mean Cl_{tot} was 11.0 (2.4) L/h and mean V_{ss} of KF was 7.0 (0.9) L at the RD.

Moreover, no accumulation was observed during course one with a geometric mean of the ratio of the AUC on day 5 to the AUC on day 1 of 1.03 and a 95% confidence interval from

Table 3 The main treatment-related hematologic adverse events per patient as a function of dose

		CTC grades				
Item	Dose level	1	2	3	4	Total no. patients
Hemoglobin (mmol/L)	20	1	0	0	0	1
	40	1	0	0	0	1
	80	0	1	0	0	1
	160	1	0	0	0	1
	320	3	1	0	0	4
	425	1	2	0	0	3
	560	3	3	0	0	6
	700	10	1	0	0	11
	930	2	0	0	0	2
Platelets (10^9/L)	20	0	0	0	0	0
	40	0	0	0	0	0
	80	1	0	0	0	1
	160	0	0	0	0	0
	320	0	0	0	0	0
	425	1	0	0	0	1
	560	1	0	0	0	1
	700	4	0	0	0	4
	930	1	0	0	0	1
Leucocytes (10^9/L)	20	0	0	0	0	0
	40	0	0	0	0	0
	80	0	0	0	0	0
	160	0	0	0	0	0
	320	0	0	0	0	0
	425	1	0	0	0	1
	560	1	0	0	0	1
	700	2	1	0	0	3
	930	0	0	0	0	0
Neutrophils (10^9/L)	20	0	0	0	0	0
	40	0	0	0	0	0
	80	0	0	0	0	0
	160	0	0	0	0	0
	320	0	0	0	0	0
	425	0	1	0	0	1
	560	1	0	0	0	1
	700	2	1	0	0	3
	930	0	0	0	0	0

Table 4 Biochemistry at all dose levels, worst per patient

		CTC grades				
Item	Dose level	1	2	3	4	Total no. patients
Creatinine (μmol/L)	20	0	0	0	0	0
	40	0	0	0	0	0
	80	0	0	0	0	0
	160	0	0	0	0	0
	320	1	0	0	0	1
	425	2	0	0	0	2
	560	2	1	0	0	3
	700	3	1	0	0	4
	930	1	0	0	0	1
Bilirubin (μmol/L)	20	0	0	0	0	0
	40	1	0	0	0	1
	80	0	0	0	0	0
	160	0	0	0	0	0
	320	1	0	0	0	1
	425	0	0	0	0	0
	560	1	0	0	0	1
	700	0	0	1	0	1
	930	1	0	1	0	2
AST (units/L)	20	0	0	0	0	0
	40	0	0	1	0	1
	80	1	0	0	0	1
	160	0	0	0	0	0
	320	2	1	0	0	3
	425	2	0	0	0	2
	560	3	2	1	0	6
	700	2	1	6	2	11
	930	0	0	3	0	3
ALT (units/L)	20	1	0	0	0	1
	40	0	1	0	0	1
	80	0	0	0	0	0
	160	0	0	0	0	0
	320	1	1	0	0	2
	425	1	1	0	0	2
	560	2	0	3	0	5
	700	0	3	3	5	11
	930	0	0	1	2	3
GGT (units/L)	20	0	0	0	0	0
	40	0	0	0	1	1
	80	1	0	0	0	1
	160	1	0	0	0	1
	320	0	0	0	0	0
	425	2	0	0	0	2
	560	3	0	1	0	4
	700	2	2	6	0	10
	930	0	1	2	0	3
AP (units/L)	20	1	0	0	0	1
	40	0	0	0	1	1
	80	0	0	1	0	1
	160	1	0	0	0	1
	320	2	2	0	0	4
	425	0	1	1	0	2
	560	2	2	1	0	5
	700	8	3	0	0	11
	930	1	0	1	0	2

Table 5 Occurrence of possibly, probably, or definitely drug-related nonhematologic toxicities at all dose levels

Item	Dose level	CTC grades 1	2	3	4	Total no. patients (%)
Gastrointestinal toxicity						
Nausea	20	0	0	0	0	0
	40	0	0	0	0	0
	80	0	1	0	0	1
	160	0	0	0	0	0
	320	0	1	0	0	1
	425	2	0	0	0	2
	560	1	0	0	0	1
	700	1	0	0	0	1
	930	0	0	0	0	0
Vomiting	20	0	0	0	0	0
	40	0	0	0	0	0
	80	0	1	0	0	1
	160	0	0	0	0	0
	320	0	1	0	0	1
	425	1	0	0	0	1
	560	1	0	0	0	1
	700	4	0	0	0	4
	930	0	0	0	0	0
Main other observed toxicities						
Fatigue	20	1	0	0	0	1
	40	0	0	0	0	0
	80	0	0	1	0	1
	160	1	0	0	0	1
	320	3	0	1	0	4
	425	0	0	1	0	1
	560	1	3	0	0	4
	700	1	5	1	0	7
	930	0	1	1	0	2
Hematuria	20	0	0	0	0	0
	40	0	0	0	0	0
	80	0	0	0	0	0
	160	0	0	0	0	0
	320	0	0	0	0	0
	425	0	0	0	0	0
	560	0	0	0	0	0
	700	0	1	0	0	1
	930	0	0	0	0	0
Hypersensitivity reactions	20	0	0	0	0	0
	40	1	0	0	0	1
	80	0	0	0	0	0
	160	0	0	0	0	0
	320	0	0	0	0	0
	425	0	0	0	0	0
	560	1	0	0	0	1
	700	5	0	1	0	6
	930	2	1	0	0	3
Hypertension	20	0	0	1	0	1
	40	0	0	0	0	0
	80	0	0	0	0	0
	160	0	0	0	0	0
	320	0	0	1	0	1
	425	0	0	0	0	0
	560	0	0	0	0	0
	700	0	0	0	0	0
	930	0	0	0	0	0

0.95 to 1.12. Interpatient variability was moderate at the dose levels with higher number of patients. For example, at 700 μg per m^2 per day where 12 patients were treated, the coefficient of variation of Cl_{tot} and V_{ss} were 34.6% and 38.2%, respectively.

Pharmacokinetics studies in urine were done only in patients treated from the moment the MTD was defined. Urine was collected over the first 2 days of KF administration. An attempt was made to measure KF concentrations in urine by liquid chromatography tandem mass spectrometry (24). However, urine concentrations of KF were below the lower limit of quantitation at all dose levels. For this reason, the cumulative urinary excretion could not be determined.

Response. All patients were evaluable for response. Five patients (16%) had stable disease. One patient had stable disease for 7 months and two patients had stable disease for 3 months. The duration of stable disease was measured from the start of the treatment until the criteria for progression were met. A decrease in PSA level, according to the criteria described in Materials and Methods, was an indication for stable disease. Twenty-six patients (81%) showed disease progression. One patient (3%) at dose level 80 μg per m^2 per day had a partial response as determined by PSA-based criteria.

Prostate-Specific Antigen. PSA is a good tumor marker to follow the effectiveness of treatment of prostate cancer and was therefore analyzed as a potential auxiliary end point. PSA values can increase dramatically in patients with prostate cancer and therefore the effect of KF treatment can be easily followed. If PSA values remain high, the therapy was probably insufficient. At dose level 80 μg per m^2 per day one patient, who had a partial remission, showed a significant decrease in PSA level (>50%) for ≥4 weeks and this was associated with clinical improvement (Fig. 5). This patient had a partial response for 5 months.

The PSADT was calculated in patients with a rising PSA during therapy. PSADT has originally been introduced to describe the natural history of prostate cancer in untreated patients who were followed conservatively (26). The PSADT in the patient with partial remission was infinite as his PSA remained stable (or decreased), and the same was observed for 3 of 26 patients with progressive disease, who had no elevations in PSA level, but were progressive due to some other adverse events. The median PSADT was 3.6 months (range, 2.2-16.3 months) in patients with stable disease and 2.3 months (range, 0.4-195 months) in patients with progressive disease, which is very similar to that in patients who progress under antiandrogen therapy alone (27).

DISCUSSION

In this clinical phase I study KF was given as an i.v. infusion over 1 hour daily for five consecutive days every

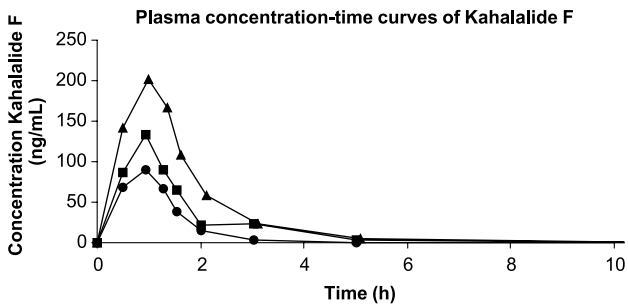

Fig. 2 Mean plasma concentration-time curves of Kahalalide F during course one in patients receiving 560 μg per m^2 per day (-●-), 700 μg per m^2 per day (-■-), and 930 μg per m^2 per day (-▲-).

Table 6 Summary of noncompartmental pharmacokinetic variables for Kahalalide F

Dose (μg/m²/d)	C_{max} (ng/mL)	AUCINF (h ng/mL)	$t_{1/2}$ (h)	Cl (L/h)	V_{ss} (L)
320 (n = 4)					
Mean	37.88	53.43	0.49	14.47	8.86
SD	12.50	27.52	0.14	6.31	1.25
CV (%)	33.01	51.50	29.32	43.61	14.09
425 (n = 3)					
Mean	58.87	82.65	0.55	12.72	9.79
SD	27.26	45.73	0.06	6.50	5.40
CV (%)	46.31	55.33	11.21	51.12	55.14
560 (n = 6)					
Mean	86.32	105.14	0.47	11.13	7.09
SD	9.39	18.32	0.11	2.35	0.92
CV (%)	10.88	17.42	24.47	21.10	12.95
700 (n = 12)					
Mean	114.18	156.83	0.58	9.41	7.64
SD	30.66	31.08	0.16	3.26	2.92
CV (%)	26.85	19.82	27.51	34.62	38.24
930 (n = 3)					
Mean	183.67	280.03	0.88	6.89	7.19
SD	29.19	70.27	0.06	1.92	2.33
CV (%)	15.90	25.09	6.24	27.82	32.42

NOTE. Variables expressed as mean (SD). Results of day 1. SD was not calculated for $n < 3$.

3 weeks in 32 patients with androgen refractory prostate cancer. The patients were treated at nine dose levels of 20 to 930 μg per m^2 per day. Based on the preclinical pharmacologic and toxicologic findings 20 μg per m^2 per day was chosen as the starting level. The daily times five schedule every 3 weeks was chosen based on the preclinical activity and toxicity data. The marine derived anticancer drugs, including KF, are known for their potencies (18). Therefore, these drugs are usually given at very low doses.

The drug was in general well tolerated. CTC grade 3 and 4 transaminase elevation was observed at the dose levels 560, 700, and 930 μg per m^2 per day. This adverse event occurred after the start of KF administration in every course and was rapidly reversible and noncumulative. No symptoms were associated to the transaminase elevations. At the dose level of 930 μg per m^2 per day, this drug-related toxicity was considered dose limiting. Therefore, this dose level was considered to be the MTD. CTC grade 3 and 4 AST and ALT elevations still occurred at the dose level of 700 μg per m^2 per day. Therefore, the optimal dose for phase II evaluation was 560 μg per m^2 per day on this schedule.

Other nonhematologic toxicities encountered were CTC grade 1 and 2 nausea, vomiting, fatigue, hematuria, and hypersensitivity reactions, which usually occurred at the higher dose levels (Table 5). The hypersensitivity reactions were drug-related. Clemastine (2 mg i.v.) was given as premedication to the patients who experienced this adverse event. Biochemical abnormalities are presented in Table 4.

The observed hematologic toxicities were CTC grade 1 to 2 anemia, thrombocytopenia and neutropenia (Table 3). None of these hematologic toxicities were clearly drug related.

KF was well tolerated at the RD (560 μg per m^2 per day) for phase II evaluation. As detailed in Tables 3 to 5, no CTC grade 4 toxicities were found. CTC grade 3 toxicities included increases in ALT and AST in three of six patients and increases in GGT and alkaline phosphatase in one of six patients, each. All other toxicities at the RD were CTC grade ≤2.

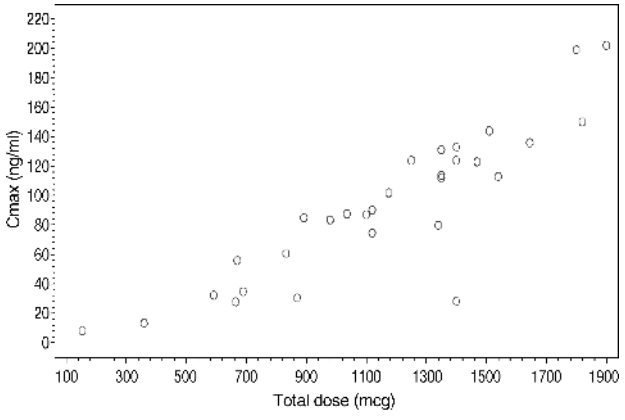

Fig. 3 Total KF dose in μg plotted against the mean C_{max} in ng/mL.

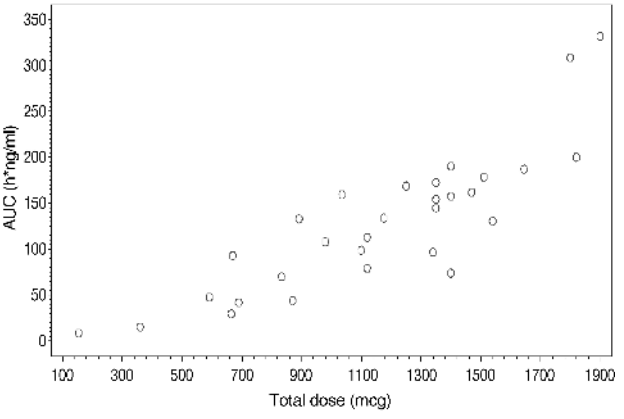

Fig. 4 The relationship between $AUC_{0-\infty}$ in h ng/L and the total dose of KF in μg.

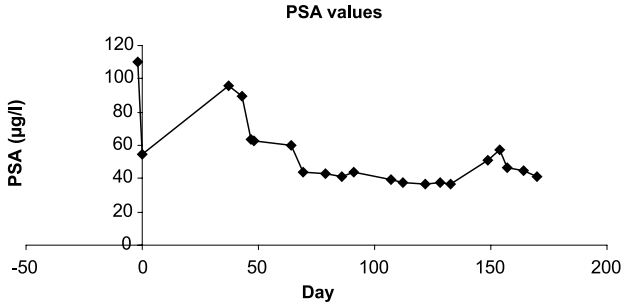

Fig. 5 The relationship between the PSA level and the duration of KF treatment in a responding patient receiving 80 µg per m^2 per day.

Pharmacokinetics monitoring was done in all patients. Plasma concentrations of KF were above the lower limit of quantitation for only up to 3 to 4 hours after the end of the infusion, because of the very low doses that were given. Moreover, a rapid elimination was observed with a mean terminal half-life of 0.47 hour (0.11 hour) at the RD. Because of this rapid elimination no accumulation of KF was observed when given daily times 5 with a ratio of the geometric mean of AUC on day 5 to AUC on day 1 of 1.03 (95% confidence interval, 0.95-1.12). Preliminary preclinical data also indicated that KF was rapidly eliminated from plasma with limited binding to extravascular tissue (28). The short terminal half-life raises the possibility to perform clinical trials to evaluate longer infusion times. With longer infusion times the activity of KF might be enhanced by prolonging tumor exposure. Graphical exploration (Figs. 3 and 4) suggest that linearity is maintained up to the RD. The higher exposure to KF at the higher dose levels, 560, 700, and 930 µg per m^2 per day, could explain the occurrence of the rapidly reversible CTC grade 3 and 4 increases in transaminases at these dose levels. This finding could not be explained by the presence of metabolites because no metabolites could be identified thus far (29). The changes in liver function, which were experienced at the higher dose levels, did not alter the pharmacokinetics of course 2 compared with the pharmacokinetics of course 1 (data not shown). Mean V_{ss} of KF at the RD was 7.0 (0.9) L and mean Cl_{tot} was 11.1 (2.3) L/h. The cumulative urinary excretion could not be determined, because of the very low KF concentrations in urine.

KF was investigated in another phase I study (KHF-A-002-01; ref. 30). Study KHF-A-002-01 investigated a continuous weekly 1-hour i.v. infusion schedule. It differed from this current study in that it included patients with advanced solid tumors in general compared with the disease-oriented design of the study reported here. In addition, a classic dose escalation design with cohorts of at least three patients was used. Dose levels were 266, 400, 530, 650, 800, 1,000, and 1,200 µg/m^2. The DLT was, as in the present study, CTC grade 3 to 4 asymptomatic reversible transaminase elevation, clearly dose-dependent, reaching the peak level 5 hours after the infusion, nonreversible by day 8 (schedule limiting) at 1,200 µg/m^2. The recommended dose for phase II was 650 µg/m^2 per week (30).

The mechanism of action of KF is currently unknown. This obviously limits the type of correlative studies that can be done to assess if the therapy with KF is acting through the presumed mechanism. Tumor cells with higher HER-2/*neu* and/or HER3 expression are particularly sensitive to the *in vitro* exposure to KF, which induces a form of caspase-independent cell death that correlates with down-regulation of Akt signaling (16). Although these results need to be validated *in vivo*, the determination of Her3 and phosphorilated Akt in patients tumor tissue obtained before treatment may be used for correlation with tumor response to treatment. Likewise, phosphorilated Akt may be determined before and after treatment in biopsy specimens obtained from patients with accessible tumor lesions to follow kahalalide F induced changes and correlate them with therapeutic outcome.

PSA is the most widely used serum marker to diagnose and monitor patients with prostate cancer. However, the correlation between serum PSA and the tumor volume is poor and the variance is high in patients with prostate carcinoma. After hormonal manipulations, the relation between tumor growth and height of the serum PSA is disturbed. The presumed mechanism for the observation that serum PSA levels in men with prostate carcinoma are significantly higher, on average, than levels in men without carcinoma is that PSA leaks from malignant cells and glands into the interstitium, then into the blood circulation instead of being confined to the ductal excretory system. In this clinical study, one would expect a decline in PSA following KF treatment. Moreover, earlier preclinical studies have identified lysosomes as the most likely intracellular target for KF based upon the evidence that KF disrupts the integrity of lysosomal membranes (10, 11). If the mechanism of action seems to be at the lysosomal level, then prostate cells in which lysosomes are very active (12–15) should be very sensitive for KF treatment. Given that this is a phase I trial and that the precise mechanisms of action of KF against the tumoral cells are not well understood, treatment duration of at least 6 weeks without any decline in PSA was allowed, to increase the knowledge on this new experimental drug. PSA-negative patients with increasing tumor related bone pain or needs for analgesia were also included in the study, because there was no clear evidence of PSA decline on this schedule. One patient had a PSA decline of >50% (Fig. 5). PSADT could also be a potential surrogate end point in hormone refractory prostate cancer. This dynamic variable could be used to assess the antiproliferative activity of cytotoxic agents and to compare the efficacy of available therapies, keeping in mind that PSA changes do not necessarily always reflect tumor behavior (31). The ultimate proof for PSADT to meet the criteria for true surrogacy can only be obtained through phase III studies where survival is the primary end point.

In conclusion, the recommended dose for KF when given as an i.v. infusion daily for 5 days every 3 weeks, is 560 µg per m^2 per day. Rapidly reversible CTC grade 3 and 4 transaminase elevations were considered to be the DLT.

REFERENCES

1. Hamann MT, Otto CS, Scheuer PJ. Kahalalides: bioactive peptides from a marine mollusk *Elysia rufescens* and its algal diet *Bryopsis* sp. J Org Chem 1996;61:6594–660.

2. Hamann MT, Scheuer PJ. Kahalalide F: a bioactive depsipeptide from the sacoglossan mollusk *Elysia rufescens* and the green alga *Bryopsis* sp. J Am Chem Soc 1993;115:5825–6.

3. Lopez-Macia A, Jimenez JC, Royo M, Giralt E, Albericio F. Synthesis and structure determination of Kahalalide F. J Am Chem Soc 2001;123: 11398–401.

4. Faircloth GT, Smith B, Grant W, et al. Selective antitumor activity of Kahalalide F, a marine-derived cyclic depsipeptide. Proc Am Assoc Cancer Res 2001;42:1140.

5. Mahler C, Verhelst J, Denis L. Clinical pharmacokinetics of the antiandrogens and their efficacy in prostate cancer. Clin Pharmacokinet 1998;34:405–17.

6. Isaacs JT. Role of androgens in prostatic cancer. Vitam. Horm 1994;49:433–502.

7. Petrylak DP, Tangen C, Hussain M, et al. SWOG 99-16: Randomized phase III trial of docetaxel (D)/Estramustine (E) versus mitoxantrone (M)/prednisone (P) in men with androgen independent prostate cancer. J Clin Oncol 2000; ASCO Annual Meeting Proc 22:2,3a.

8. Eisenberger MA, De Witt R, Berry W, et al. A multicenter phase III comparison of docetaxel + prednisone and mitoxantrone + prednisone in patients with hormone-refractory prostate cancer. J Clin Oncol 2000, ASCO Annual Meeting Proc 22:2,3a,4a.

9. Faircloth G, Sheuer P, Avila J, Hendricks H, Drees M, Jimeno J. Kahalalide F, a new marine depsipeptide with selective activity against solid tumor models. Ann Oncol 1996;7:33.

10. Garcia-Rocha M, Bonay P, Avila J. The antitumor compound Kahalalide F acts on cell lysosomes. Cancer Letters 1996;99:43–50.

11. Faircloth G, Grant W, Smith B, et al. Preclinical development of Kahalalide F, a new marine compound selected for clinical studies. Proc Am Assoc Cancer Res 2000;41:3823.

12. Brandes D. The fine structure and histochemistry of prostatic glands in relation to sex hormones. Int Rev Cytol 1996;20:207–76.

13. Brandes D, Boume G. Histochemistry of some aspects of prostate pathology. Alt Cancer Inst Monogr 1963;12:29–41.

14. Gunn S, Gould T. Hormonal interrelatioship affecting the selective uptake of ZN65 by the dorsolateral prostate of the hypophysectomized rat. J Endocrinol 1956;16:18–27.

15. Hanauske AR, Hanauske U, Von Hoff DD. The human tumor clonogenic assay in cancer research and therapy. Curr Probl Cancer 1985;9:1–66.

16. Janmaat ML, Kruyt FA, Jimeno JM, Rodriguez JA, Giaccone G. Kahalalide F (KF) induces caspase-independent cytotoxicity that correlates with HER2/*neu* and/or HER3 expression levels and is accompanied by down-regulation of Akt signalling. Proc Am Assoc Cancer Res 2004;5328a:110.

17. Brown AP, Morrissey RL, Faircloth GT, Levine BS. Preclinical toxicity studies of Kahalalide F, a new anticancer agent: single and multiple dosing regimens in the rat. Cancer Chemother Pharmacol 2002;50:333–40.

18. Jimeno JM, Faircloth G, Cameron L, et al. Progress in the acquisition of new marine-derived anticancer compounds: development of ecteinascidin-743 (ET-743). Drugs of the future 1996;21: 1155–65.

19. Nuijen B, Bouma M, Talsma H, et al. Development of a lyophilized, parenteral pharmaceutical formulation of the investigational polypeptide marine anticancer agent Kahalalide F. Drug Dev Ind Pharm 2001;27: 767–80.

20. Ontario PSA Clinical Guidelines. The PSA Clinical Guideline Expert Committee for the Laboratory Proficiency Testing Program (LPTP), 97.09.30:Physician Reference Document.

21. Bubley GJ, Carducci M, Dahut W, et al. Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations for the Prostate-Specific Antigen Working Group. J Clin Oncol 1999;17:3461–7.

22. Scher HI, Kelly WMK, Zkang ZF, et al. Post-therapy serum prostate-specific antigen level and survival in patients with androgen-independent prostate cancer. J Natl Cancer Inst 1999;91:244–51.

23. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment of cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.

24. Guidelines for reporting of adverse drug reactions. Division of Cancer treatment. Bethesda (MD): National Cancer Institute; 1988.

25. Stokvis E, Rosing H, Lopez-Lazaro L, et al. Quantitative analysis of the novel depsipeptide anticancer drug Kahalalide F in human plasma by high-performance liquid chromatography under basic conditions coupled with positive turbo-ionspray tandem mass spectrometry. J Mass Spectrom 2002;37:992–1000.

26. Schmid HP, Morant R, Bernhard J, Maibach R. Prostate specific antigen doubling time as auxiliary end point in hormone refractory prostatic carcinoma. Eur Urology 2003;43:28–30.

27. Fowler JE, Pandey P, Seaver LE. Prostate specific antigen regression and progression after androgen deprivation for localized and metastatic prostate cancer. J Urol 1995;153:1860–5.

28. Supko JG, Lu H, Jimeno JM, Grant W, Faircloth GT. Preclinical pharmacology studies with the marine natural product Kahalalide F. Clin Cancer Res 1999;5:315.

29. Sparidans RW, Stokvis E, Jimeno JM, et al. Chemical and enzymatic stability of a cyclic depsipeptide, the novel, marine-derived anticancer agent Kahalalide F. Anticancer Drugs 2001;12:575–82.

30. Ciruelos E, Trigo JM, Pardo B, et al. A phase I and pharmacokinetic study with Kahalalide F in patients with advanced solid tumors using a continuous weekly one hour intravenous infusion schedule. Eur J Cancer 2002;38:95a.

31. Schroeder FH, Norming U, Blumenstein BA. Phase II studies on prostate cancer. Urology 1997;49:3–14.