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Phase I and pharmacokinetic study of continuous twice weekly intravenous administration of Cilengitide (EMD 121974), a novel inhibitor of the integrins αvβ3 and αvβ5 in patients with advanced solid tumours

F.A.L.M. Eskens[a,*], H. Dumez[b], R. Hoekstra[a], A. Perschl[c], C. Brindley[d], S. Böttcher[c], W. Wynendaele[b], J. Drevs[e], J. Verweij[a], A.T. van Oosterom[b]

[a]*Erasmus Medical Center, Department of Medical Oncology, PO Box 2040, 3000 CA Rotterdam, The Netherlands*
[b]*University Hospital Gasthuisberg, 3000 Leuven, Belgium*
[c]*Merck KGaA, D-64293 Darmstadt, Germany*
[d]*Quintiles Scotland Limited, Edinburgh EH14 4AP, Scotland, UK*
[e]*Tumour Biology Center, Freiburg, Germany*

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Abstract

A single-agent dose escalating phase I and pharmacokinetic study with Cilengitide, an inhibitor of the integrins αvβ3 and αvβ5, was performed to determine its safety and toxicity. Cilengitide was administered as a one-hour infusion twice weekly without interruption to patients with histologically- or cytologically-confirmed metastatic solid tumours. Plasma pharmacokinetics were determined at days 1 and 15. 37 patients were enrolled into the study. Dose levels studied were 30, 60, 120, 180, 240, 400, 600, 850, 1200, and 1600 mg/m^2/infusion. There was no dose-limiting toxicity (DLT). Pharmacokinetics were dose-independent and time-invariant. Apparent terminal half-life ranged from 3 to 5 h. At 120 mg/m^2/infusion, peak plasma concentrations were attained that optimally inhibited tumour growth in preclinical models. Cilengitide can be safely administered using a continuous twice-weekly infusion regimen. As DLT was not reached, future trials should explore Cilengitide at different doses.

Keywords: Phase I clinical trial; Angiogenesis inhibitor; Integrins; Cilengitide (EMD 121974); Pharmacology

1. Introduction

Angiogenesis is a strictly regulated process with only localised and temporary bursts of activity under physiological conditions like hair growth, the female reproductive cycle and wound repair. In tumour-related angiogenesis, a more chronic *pro*-angiogenic phenotype is found, resulting from an emerging imbalance between *pro*-angiogenic and *anti*-angiogenic stimuli. Vascular Endothelial Growth Factor (VEGF) and basic Fibroblast Growth Factor (bFGF) are *pro*-angiogenic factors that can be released by growing tumours.

Newly formed tumour-related blood vessels sprout into the extracellular matrix (ECM), a process dependent on the ability of proliferating endothelial cells to interact with diverse glycopotein components of this ECM. This interaction is mediated by endothelial transmembrane receptors or integrins αvβ3 and αvβ5 [1–3]. Whereas quiescent cells hardly express integrins, proliferating endothelial cells are the primary source of increased αvβ3 expression found in various human epithelial tumours [3–5].

In *in vivo* angiogenesis models, αvβ3 and αvβ5 expression has been found to be correlated with the *pro*-angiogenic factor predominantly involved; αvβ5 expression is upregulated by VEGF, whereas bFGF stimulates the upregulation of αvβ3 [6–8]. Increased expression of endothelial αvβ3 has been found to have major prognostic impact in patients with breast carcinoma [9].

* Corresponding author. Tel.: +31-10-463-4897; fax: +31-10-463-4627.
E-mail address: f.eskens@erasmusmc.nl (F.A.L.M. Eskens).

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Inhibition of tumour-related angiogenesis has been a target for anticancer drug development for several years now, and various different approaches are currently being explored. Anti-angiogenic treatment by blocking endothelial integrin activity seems to be an attractive approach, and monoclonal antibodies directed against αvβ3 as well as low-molecular-mass peptides blocking αv or vitronectin receptors have been developed.

Cilengitide (EMD 121974, Cyclo-L-Arg-Gly-L-Asp-D-Phe-N (Me) L-Val, Merck KGaA, Darmstadt, Germany) is an example of such a low-molecular-mass peptide (MW 588.7 g/mol) (Fig. 1). It has an IC_{50} for the inhibition of αvβ3 receptor binding to vitronectin of 1 nM, whereas the IC_{50} for inhibiting the αvβ5 receptor binding to vitronectin was 140 nM. Cilengitide does not block the αIIbβ3 fibrinogen receptor, which is the receptor for inhibitors of platelet aggregation, and exerts no anti-aggregatory activity *in vitro* in concentrations up to 10 μM.

In vitro studies with Cilengitide using a chicken chorioallantoic membrane model seeded with the M21-L human melanoma lacking the αv-chain, and UCLA-P3 human lung carcinoma and FgM human pancreatic carcinoma, both lacking the αvβ3 receptor, showed inhibition of angiogenesis mediated locally in the vascular beds of the tumours and tumour regression. A direct effect of Cilengitide on the tumours was unlikely because of the absence of the full receptor on these cells.

In vivo studies of EMD 85189 (the hydrochloride salt of Cilengitide) given twice daily, once daily or every other day with M21-L human melanoma xenografts showed profound inhibitory effects on tumour growth and size. The optimal inhibitory activity on tumour growth in nude mice bearing M21-L human melanoma xenografts was seen at a dose of 10–15 mg/kg, yielding peak plasma concentrations (C_{max}) of 10.9–12.7 μg/ml.

Toxicology studies in rodents showed lateral and abdominal position, tonic-clonic convulsions, locomotion disturbance, and dyspnoea after single high-dose intravenous (i.v.) administrations in acute toxicity studies. Four weeks daily i.v. bolus administrations in mice showed no evidence of treatment-related effects on any of the parameters investigated up to and including the highest dose levels of 90 mg/kg. Four-week toxicology studies with daily i.v. bolus injections in cynomolgus monkeys at doses up to 90 mg/kg showed dose-related anaemia and reticulocytosis at the end of the study period with normalisation of both parameters after 4 weeks. Necropsy and histopathology revealed no bone marrow abnormality explaining the anaemia.

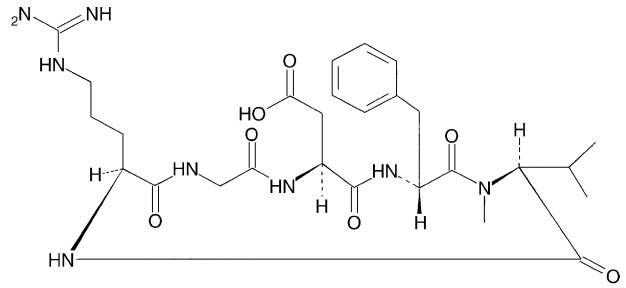

Fig. 1. Chemical structure of Cilengitide (EMD 121974).

We performed the first phase I and pharmacokinetic study with Cilengitide in patients with advanced solid tumours, using a continuous twice-weekly i.v. treatment schedule.

2. Patients and methods

2.1. Eligibility criteria

Patients with a histologically- or cytologically-confirmed diagnosis of solid tumour refractory to standard treatment or for which no standard therapy was available were eligible for the study. Further eligibility criteria included: age ≥ 18 years; Karnofsky Performance Status ≥ 70%; life expectancy ≥ 12 weeks; no major surgery or anticancer therapy in the previous 4 weeks (6 weeks for nitrosoureas, mitomycin C and melphalan); adequate function of bone marrow (neutrophils $\geq 2.0\times10^9$ cells/l, haemoglobin (Hb) ≥ 6.2 mmol/l, platelets $\geq 100\times10^9$ cells/l); adequate hepatic function (bilirubin < 1.5 × upper normal limit, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) < 3 × upper normal limit or < 6 × upper normal limit in case of liver metastases); adequate renal function (serum creatinine < 1.5 × upper normal limit); no history of HIV, active Hepatitis C or Hepatitis B infections; no organ allografts other than autologous bone marrow transplantation after high dose chemotherapy; no clinical evidence of central nervous system (CNS) metastases.

Specific exclusion criteria were menstruating female patients; clinical relevant fluid effusions requiring treatment; unstable cardiac conditions requiring treatment; diabetes mellitus requiring treatment; coagulation disorders as defined by International Normalized Ratio (INR) > 1.5, prothrombin time > 18 s or Activated Prothrombin Time (APTT) > 60 s, or patients on anticoagulant therapies; hypercalcaemia (> 2.88 mmol/l); a proven history of gastric or duodenal ulcer or gastrointestinal (GI) blood loss in the last 6 weeks prior to the start of treatment, or patients at high risk of GI ulceration due to high non-steroidal anti-inflammatory drug (NSAID) consumption of > 2.4 g of aspirin-equivalent per day. Local ethics boards in both institutions approved the study. All patients gave their written informed consent.

2.2. Pretreatment assessment and follow-up studies

Prior to treatment, a complete medical history was taken and a physical examination was performed. A complete blood count (CBC) including white blood cell differential, INR and APTT, and serum chemistries including sodium, potassium, calcium, phosphorus, blood urea nitrogen, creatinine, total protein, albumin, glucose, alkaline phosphatase, bilirubin, ASAT, ALAT, gamma-glutamyl transpeptidase (GT), and lactate dehydrogenase was performed, as were urinalysis, electrocardiogram (ECG), tumour markers as appropriate, and chest X-ray. Weekly evaluations included history, physical examination, and toxicity assessment according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) [10], CBC, serum chemistries and urinalysis. Tumour measurements were performed every 4 weeks for 2 months and thereafter every 8 weeks and were evaluated according to the World Health Organization (WHO) criteria for response [11]. In cases of disease progression, patients were taken off the study.

2.3. Drug administration

Cilengitide was supplied in 30-ml vials as a clear solution at a concentration of 15 mg/ml. It was stored under refrigerated conditions at temperatures between 2 and 8 °C protected from sunlight. Before administration, the calculated dose of Cilengitide was diluted in isotonic sodium chloride, making a total volume of 250 ml. The diluted product had to be used within 24 h after preparation and was administered as a 1-h infusion using a standard infusion device. Antiemetics were not given routinely. The first 3 patients enrolled in this study were given one single infusion after which they were clinically observed for 3 consecutive days. Fourteen days after this single infusion, and after results of the pharmacokinetic analysis had become available and toxicity was assessed, treatment was continued in a twice-weekly treatment cycle. Subsequent patients started in the twice-weekly treatment cycle.

2.4. Dosage and dose escalation

The starting dose was 30 mg/m^2. This dose approximated 1/10 of the LD_{10} in mice (dose that was lethal to 10%), being 90 mg/kg or 270 mg/m^2. Dose escalation was based on pharmacokinetic results and toxicities encountered, with the maximum increase in the dose being a dose doubling. After the pharmacokinetic results of the first dose level had become available, the second dose level was selected to predictably result in 50% of the target C_{max} (i.e. 5.45 μg/ml). The third dose level was selected to result in the target C_{max}, whereas the fourth and fifth dose level were selected to predictably result in 150% and 200% of the target C_{max}, respectively. Starting with the sixth dose level, and in the absence of toxicity, planned subsequent escalation steps were 67, 50, 40, 40 and 33% increments (modified Fibonacci). In cases of drug-related toxicity, dose escalation steps would be 50% of the planned increase in dose level. If at any dose level 1 patient experienced dose-limiting toxicity (DLT), a total of 6 patients had to be recruited at that dose. DLT was considered to be any worsening of a laboratory parameter by $\geqslant 3$ CTC grades compared with the pretreatment value other than grade 3 or 4 haematological toxicity, unless this was lasting >7 days or was accompanied by fever, any clinical grade 3 or 4 adverse event that was considered to be related to treatment, grade 2 cardiac or renal toxicity, and any grade 2 mucositis, diarrhoea with blood loss or skin toxicity where delayed wound healing was considered. No intrapatient dose escalation was allowed.

2.5. Pharmacokinetic studies

For the pharmacokinetic analysis of Cilengitide, 5-ml blood samples were taken from the arm opposite to the infusion arm prior to the first drug administration, at 30 min (mid of the infusion), at 60 min (end of the infusion), at 1.25, 1.5, 2, 3, 4, 8, 10 and 24 h after the start of the infusion on days 1 and 15. On days 4 and 8, an additional 5-ml blood sample was taken predose. Of the 3 patients in the first single dose cohort of the study, an additional blood sample was taken 32 h after the start of their first infusion on day 1. Blood samples were collected in lithium-heparin-containing tubes, and were centrifuged immediately after drawing at 3000 rpm at 4 °C for 10 min, after which at least 2 ml of plasma was transferred into two plastic tubes that were stored at −20 °C until shipment. For analysis, a High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) using a Sciex API-300 series mass spectrometer fitted with a turboionspray ionization source (Perkin-Elmer Ltd, Beaconsfield, UK) was used. The lower limit of quantitation for the plasma assay was 5 ng/ml, with a linear range from 5 to 5000 ng/ml, the lower limit of quantitation for the urine assay was 0.1 μg/ml, with a linear range from 0.1 to 100 μg/ml. For each patient, C_{max} and the time of occurrence (t_{max}) were the observed values. The area under the plasma concentration versus time curve (AUC) was calculated by the log-linear trapezoidal rule and extrapolated to infinity by linear regression analysis. The total body clearance (CL) was calculated as dose/AUC. The apparent volume of distribution at steady state (V_{ss}) was calculated as dose*AUMC/$(AUC)^2$−[T/2], where AUMC is the area under the first moment curve to infinity and T is the duration of the infusion. The apparent terminal half-life ($t_{1/2}$) was calculated by dividing $\log_e 2$ by the elimination rate constant (λ_z),

estimated by linear-regression analysis of the final data points of the linear portion of the log-linear concentration–time plot. A non-linear power model was used to assess dose proportionality [12]. The proportional relationship between each parameter and dose is written as a power function:

$$\text{parameter} = a * \text{dose}^b \quad (1)$$

where a is a constant, b is the proportionality constant and parameter is the parameter of interest, i.e. AUC and C_{max}. AUC and C_{max} were each plotted against dose. The exponent, b, was the estimated slope of the resulting regression line since taking logs of Eq. (1) gives the linear relationship, log parameter = log a + b*log dose. The relationship is dose proportional when $b = 1$.

2.6. Pharmacodynamic studies

For pharmacodynamic analysis of Cilengitide, 5-ml blood samples were taken at baseline, at day 29, and after each additional 4-week cycle. An exploratory analysis was performed on serum levels of VEGF as well as on markers of endothelial cell proliferation. For this, serum levels of soluble TIE-2 (sTIE-2) or Tek, a transmembrane angiopoietin receptor tyrosine kinase uniquely expressed on endothelial cells [13,14], soluble E-Selectin [15] (sE-Selectin) and soluble *fms*-like tyrosine kinase (sFLT-1), the receptor of VEGF, were used. Serum levels of VEGF were measured using an enzyme-linked immunosorbent assay (ELISA) method as previously described in Ref. [16]. Serum levels of sE-Selectin were measured using a commercially available sandwich ELISA assay (R&D Systems, catalog number BBE 2B). Serum levels of sFLT-1 were measured using a sandwich ELISA as previously described in Ref. [17]. Serum levels of sTIE-2 were measured using a sandwich ELISA assay using mouse monoclonal antibody alpha-Tek9 as detection antibodies.

3. Results

37 patients were enrolled into this study, all of which were evaluable for toxicity. Patients' characteristics are given in Table 1. Dose levels studied were 30 mg/m^2 ($n = 3$), 60 mg/m^2 ($n = 3$), 120 mg/m^2 ($n = 3$), 180 mg/m^2 ($n = 3$), 240 mg/m^2 ($n = 3$), 400 mg/m^2 ($n = 3$), 600 mg/m^2 ($n = 4$), 850 mg/m^2 ($n = 3$), 1200 mg/m^2 ($n = 6$), and 1600 mg/m^2 ($n = 6$). At 1200 mg/m^2, 3 additional patients were entered due to the occurrence of a fatal lung embolism that was unlikely to be related to the agent. At 1600 mg/m^2, 6 patients were entered in order to obtain additional safety and pharmacokinetic data.

3.1. Premature study discontinuation

3 patients discontinued the study prematurely; 1 patient at 120 mg/m^2 discontinued after 19 days due to rapid disease progression. One patient with colorectal carcinoma treated at 240 mg/m^2 discontinued after 15 days due to recurrent bowel obstruction. One patient treated at 1200 mg/m^2 discontinued treatment after 18 days and died 1 day later. At autopsy, he was found to have extensive pulmonary embolisms. No patients discontinued treatment prematurely due to drug-related toxicity.

3.2. Haematological toxicity

There was no drug-related neutropenia or thrombocytopenia. One patient at 400 mg/m^2 had grade 1 leucopenia at the start of treatment related to their previous treatment. With ongoing treatment, this leucopenia subsided.

Anaemia never exceeded grade 2 and in all cases was considered to be disease-related.

3.3. Non-haematological toxicity

Non-haematological toxicity was mild, never exceeding grade 2, and consisted of nausea, anorexia, vomiting, fatigue and malaise. There was no hyperglycaemia, renal or hepatic toxicity. There was no tendency to more frequent, more intense or more prolonged toxicity with either prolonged treatment or increasing doses. Headache up to grade 2 was recorded in 2 patients at 600 and 1200 mg/m^2, respectively. One patient at 1200 mg/m^2 developed a skin rash grade 2 on the trunk and

Table 1
Patients' characteristics

No. of patients entered	37
No. of patients evaluable	37
Male/female	25/12
Median age (years)	55
Range (years)	39–81
Median Karnofsky performance score	90%
60%	1
70%	3
80%	8
90%	11
100%	13
Unknown	1
Primary tumour site	
Renal cell	9
Colorectal	8
Unknown primary	3
Melanoma	2
Non small cell lung	2
Cervix	2
Head and neck	2
Miscellaneous	9

back in the fifth week of treatment. A punch biopsy from a lesion revealed the classical picture of toxic dermatitis. After discontinuation of treatment 28 days later, when disease assessment had revealed disease progression, the skin abnormalities gradually disappeared. Another patient at 1200 mg/m^2 suddenly died after six administrations of Cilengitide. Multiple pulmonary embolisms were found on autopsy. A relationship between the cause of death and the trial drug was considered to be unlikely. This patient with advanced non-small cell lung cancer had a KPS of 60% when enrolled into the study and was thus actually ineligible. The lethal embolism was considered to be disease-related. However, it was due to this event that at the dose level of 1200 mg/m^2 3 additional patients were enrolled. At this dose level, and at the subsequent and highest dose level, no additional thromboembolic complications were seen. There were no data suggestive of impaired wound healing.

3.4. Pharmacokinetic analysis

Mean (standard deviation (S.D.)) pharmacokinetic parameters following single (day 1) and repeated (day 15) infusions of Cilengitide are summarised in Tables 2 and 3. C_{max} was generally observed at the end of the infusion period. Mean systemic clearance (CL) was 34 to 66 ml/min/m^2 and apparent volume of distribution (V_{ss}) was 9–17 l/m^2, manifested as a terminal half-life of 3–5 h. Mean plasma concentration-time profiles following single (day 1) and repeated (day 15) infusions are given in Figs. 2 and 3. Figs. 4 and 5 summarise the relationship between dose and mean C_{max} and the mean AUC following single (day 1) and repeated (day 15) infusions, respectively. The exponent of the power function fitted to the mean C_{max} and AUC data was close to unity, indicating that systemic exposure to Cilengitide increased in a dose-proportional manner.

3.5. Pharmacodynamic analysis

Serum levels of VEGF, sFLT-1, sTIE-2, and sE-Selectin were measured on days 1 and 29 (data not shown separately). The mean plus S.D. and median percentage change in these levels following 28 days of treatment in patients with either progressive or stable disease are shown in Table 4. Although no significant differences in the serum levels of any of the markers in patients with either stable or progressive disease could be determined, a tendency for correlation between serum concentrations of sFLT-1, sTIE-2 and VEGF and response to treatment was seen.

3.6. Responses

There were no partial or complete responses. Prolonged stable disease was seen in 2 patients with renal

Table 2
Mean (S.D.) pharmacokinetic parameters of Cilengitide following a 1-h i.v. infusion (day 1)

	Dose (mg/m^2)											
Parameter:	30[a]	30	60	120	180	240	400	600	850	1200	1600	
N:	3	3	3	3	3	3	3	4	3	6	6	
C_{max} (ng/ml)	3744 (496)	3647 (230)	6589 (544)	12,142 (1903)	22,189 (4709)	26,751 (5774)	40,420 (9137)	65,410 (11941)	79,904 (12656)	115,701 (25169)	172,149 (30041)	
t_{max} (h)[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
AUC (ng.h/ml)	12,541 (3224)	12,272 (1621)	20,494 (359)	31,094 (6804)	85,101 (8421)	86,309 (30908)	160,716 (22414)	191,595 (21365)	345,409 (223706)	453,053 (167188)	551,074 (106853)	
λ_z (/h)	0.195 (0.024)	0.200 (0.028)	0.195 (0.031)	0.236 (0.014)	0.152 (0.023)	0.219 (0.023)	0.181 (0.014)	0.193 (0.027)	0.194 (0.090)	0.204 (0.035)	0.223 (0.036)	
$t_{\frac{1}{2}}$ (h)	3.59 (0.42)	3.52 (0.51)	3.62 (0.63)	2.94 (0.17)	4.64 (0.77)	3.19 (0.35)	3.84 (0.31)	3.65 (0.54)	4.44 (2.80)	3.48 (0.58)	3.17 (0.44)	
CL (ml/min/m^2)	41.6 (9.9)	41.0 (5.2)	48.9 (0.7)	66.2 (13.7)	35.5 (3.5)	50.7 (18.1)	42.0 (5.8)	47.9 (12.3)	52.3 (26.7)	48.5 (19.8)	49.9 (11.2)	
V_{ss} (ml/m^2)	9895 (814)	9521 (661)	10,985 (912)	10,962 (1135)	11,990 (3354)	9624 (729)	11,615 (2174)	10,149 (1729)	11,850 (1338)	10,318 (1264)	10,590 (2022)	

C_{max}, peak plasma concentrations; AUC, area under the plasma concentration versus time curve; λ_z, elimination rate constant; $t_{\frac{1}{2}}$, terminal half life; S.D., standard deviation; CL, mean systemic clearance; V_{ss}, apparent volume of distribution.

[a] Single infusion.

[b] Median.

Table 3
Mean (S.D.) pharmacokinetic parameters of Cilengitide following a 1-h i.v. infusion (day 15)

	Dose (mg/m^2)										
Parameter:	30	60	120	180	240	400	600	850	1200	1600	
N:	3	3	3	3	3	3	4	3	6	6	
C_{max} (ng/ml)	3334 (574)	7576 (309)	11,101 (780)	19,912 (6394)	30,303 (5368)	43,418 (7777)	59,487 (8611)	76,067 (7968)	123,902 (25264)	161,619 (16937)	
t_{max} (h)[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.13	1.25	1.00	1.00	
AUC (ng.h/ml)	11,533 (3097)	21,201 (1389)	32,406 (8761)	89,065 (8197)	91,570 (19621)	161,051 (33465)	168,674 (36028)	325,720 (216631)	524,688 (198923)	521,472 (131993)	
λ_z (/h)	0.199 (0.023)	0.201 (0.011)	0.182 (0.039)	0.145 (0.028)	0.212 (0.022)	0.190 (0.009)	0.201 (0.036)	0.183 (0.059)	0.178 (0.056)	0.226 (0.038)	
$t_{\frac{1}{2}}$ (h)	3.51 (0.39)	3.46 (0.19)	3.95 (0.98)	4.92 (1.03)	3.30 (0.35)	3.66 (0.17)	3.56 (0.74)	4.15 (1.66)	4.48 (2.31)	3.14 (0.50)	
CL (ml/min/m^2)	45.0 (10.3)	47.1 (2.8)	65.1 (18.1)	34.2 (2.7)	45.2 (8.6)	63.6 (42.4)	61.5 (11.7)	54.9 (26.3)	42.7 (18.5)	55.0 (21.3)	
V_{ss} (ml/m^2)	10,392 (1822)	9988 (793)	13,938 (1748)	12,717 (4657)	9218 (958)	16,979 (11306)	12,394 (2025)	12,786 (2020)	11,025 (4078)	11,558 (1751)	

C_{max}, peak plasma concentrations; AUC, area under the plasma concentration versus time curve; λ_z, elimination rate constant; $t_{\frac{1}{2}}$, terminal half life; S.D., standard deviation; CL, mean systemic clearance; V_{SS}, apparent volume of distribution.
[a] Median.

cell carcinoma (164, 164 days) and 1 patient with colorectal carcinoma (168 days), respectively.

4. Discussion

We have performed the first phase I and pharmacokinetic study to explore the safety and tolerability of Cilengitide, a novel low-molecular-mass peptide inhibitor of the integrins αvβ3 and αvβ5. In the current study, Cilengitide could be administered safely, with no drug-related haematological toxicity and only mild drug-related non-haematological toxicity, mainly consisting of gastrointestinal and constitutional complaints. Non-haematological toxicity never exceeded grade 2 and there was no indication of increasing toxicity with either increasing dose or prolonged exposure. No DLT was recorded following a more than 50-fold dose escalation, and therefore it was not possible to define a maximum tolerated dose (MTD) or one single recommended dose for further activity testing. For non-cytotoxic drugs like Cilengitide, however, it has been well accepted that defining such a single recommended dose for further activity testing often cannot be based on defining endpoints like MTD [18,19]. Pharmacokinetic analysis revealed that plasma concentrations yielding tumour growth inhibition in animal models were reached at a dose level of 120 mg/m^2, and that 200% of this concentration was reached between 180 and 240 mg/m^2. Further analysis showed a proportional increase in mean drug exposure and C_{max} with increasing dose. The pharmacokinetic parameters of systemic clearance, apparent volume of distribution at steady state and terminal plasma half-life were not appreciably different following increasing dose levels and were similar on days 1 and 15, indicating that the kinetics were dose-independent and time-invariant within the dose range 30–1600 mg/m^2. The apparent volume of distribution was relatively small, nominally equivalent to the extracellular fluid volume. Systemic clearance of Cilengitide was low compared with hepatic and renal blood flow and lower than the glomerular filtration rate. This resulted in a relatively short plasma half-life. Therefore, and although relevant plasma concentrations were easily reached, due to the short terminal plasma half-life, a twice-weekly treatment schedule might not be optimal to yield continuous drug exposure, and different treatment schemes resulting in continuous drug exposure should therefore be explored. Using continuous i.v. infusion can, for example, attain constant plasma levels at or above the assumed active concentrations. Such treatment schedules, however, can only be administered at the cost of patient inconvenience. On the other hand, however, it should be emphasised that from animal experiments with Cilengitide we know that a pulsed treatment schedule resulted in marked tumour growth

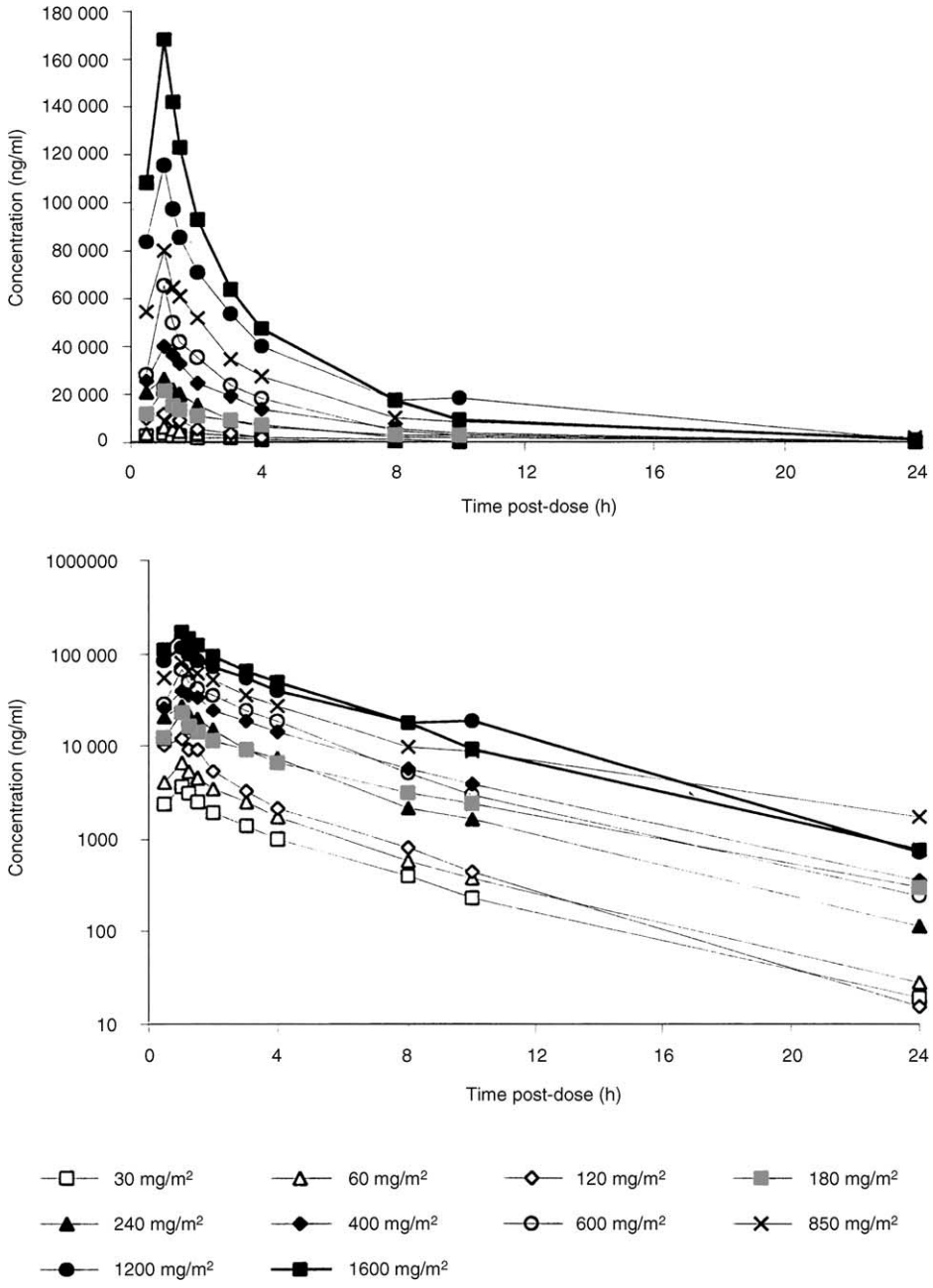

Fig. 2. Mean plasma concentrations of Cilengitide (day 1).

Table 4
Mean (S.D.) and median change (%) in serum concentrations, compared with baseline, of VEGF, sFLT-1, sTIE-2 and sE-Selectin following 28 days of treatment in patients (N) with progressive disease (PD) or stable disease (SD)

	VEGF		sFLT-1		sTIE-2		sE-Selectin	
	PD	SD	PD	SD	PD	SD	PD	SD
N	11	16	8	14	9	14	8	14
Mean (S.D.)	385.2 (581.3)	230.6 (355.9)	−5.6 (37.5)	21.6 (117.3)	66.5 (93.6)	15.6 (43.8)	17.8 (24.6)	10.9 (18.8)
Median	163.7	59.84	7.32	3.86	25.9	8.75	15.71	9.21

VEGF, vascular endothelial growth factor; sFLT-1, soluble fms-like tyrosine kinase; sE-Selectin, soluble E-Selectin; sTIE-2, soluble TIE-2.

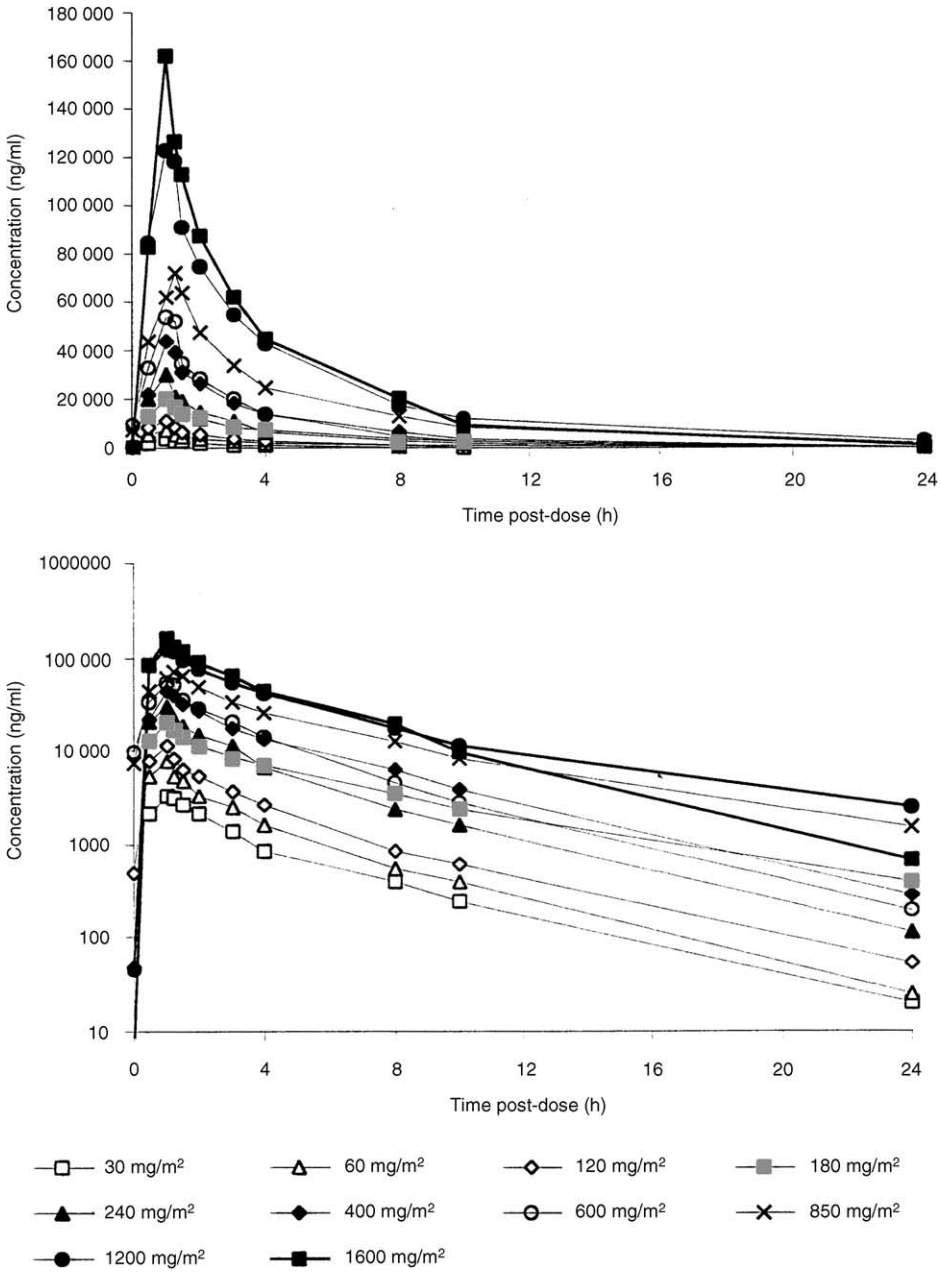

Fig. 3. Mean plasma concentrations of Cilengitide (day 15).

inhibition. The half-life of EMD 121974 in mice is approximately 10–15 min, and taking the daily administration as a basis, about 80 half-lives lie between each dose. When comparing this to the situation in humans, 20 half-lives lie between two doses at the investigated twice weekly schedule (data not shown). Thus, based on these considerations even less extensive administration schedules like once weekly could be an option.

As no tumour biopsies were taken in this study, an exploratory analysis of serum levels of various markers of endothelial cell proliferation was performed. If any dose-dependent change in marker concentration occurred, then this marker could possibly indicate an inhibitory effect of Cilengitide on endothelial cell proliferation. If, additionally, a change in the serum concentration in any of these markers could be correlated with clinical outcome, then this marker could possibly be used as a surrogate marker of antitumour activity. However, in the current study serum levels of sTIE-2, sE-Selectin, sFLT-1 and VEGF all failed to show a significant correlation with the administered dose and response following 28 days of treatment, although a tendency towards correlation was seen for some of them. These obviously disappointing results are partly explained by the limited number of blood samples that could be analysed as well as by the hetero-

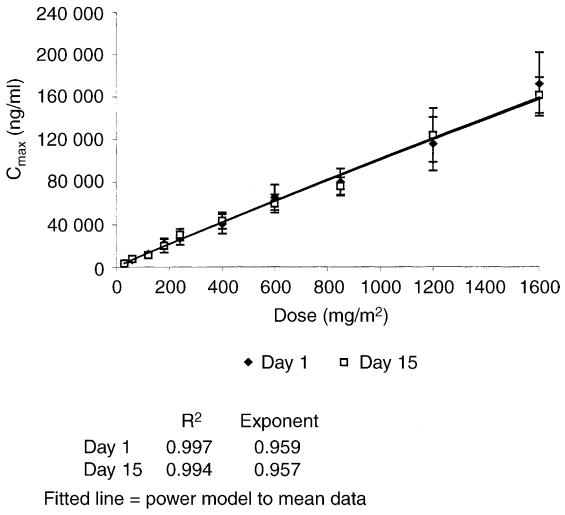

Fig. 4. Relationship between dose and C_{max} of Cilengitide (days 1 and 15).

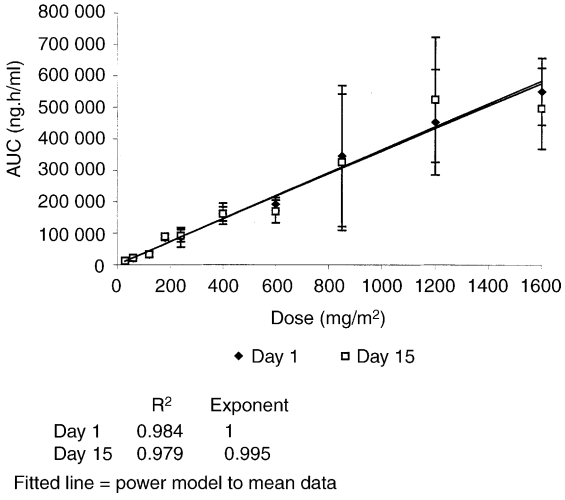

Fig. 5. Relationship between dose and AUC of Cilengitide (days 1 and 15).

geneous patient population. As the current study is the first one to report on a low-molecular-mass peptide antagonist of integrins, and only one other clinical study exploring an inhibitory monoclonal antibody to the integrin αvβ3 has been published to date without data on biological activity [20], no database indicating which surrogate marker of activity of integrin inhibitors should be used is yet available.

Based on the relative lack of toxicity, and the lack of adequate surrogate markers for the activity of Cilengitide, we are faced with the dilemma of selecting a dose for further activity testing. Using the current treatment schedule, the most practical design would be to take a low and a high dose, for example, a dose resulting in plasma levels at the effective animal concentration and one exceeding this concentration and to do a randomised study, using appropriate endpoints [21,22]. Whether these studies should be performed using single agent Cilengitide or the combination of cytotoxic chemotherapy and Cilengitide is under consideration.

Currently, the way to assess anti-angiogenic activity of the various kinds of angiogenesis inhibitors is a major challenge. In fact, a direct analysis of microvessel density in repeated tumour biopsies taken during, preferably prolonged, treatment with angiogenesis inhibitors is considered to be the most precise way to assess these effects, but as these procedures are painful and cumbersome to patients, they should be avoided. Moreover, there would be a risk of sampling error, obscuring the results. Indirect measurements of tumour blood flow by means of dynamic-enhanced magnetic resonance imaging (MRI) and/or fluorodeoxyglucose positron emission tomography (PET) are currently considered to be potentially useful techniques, but are not yet fully validated.

Inhibiting tumour-related angiogenesis can theoretically be achieved through various mechanisms of action. As VEGF currently seems to be the predominant pro-angiogenic factor, blocking VEGF prior to its attachment to the endothelial cell receptor by monoclonal antibodies could be a rational approach. Recently, two clinical studies exploring this approach have been published, showing the safety and tolerability of these antibodies, either as single agents or in combination with cytotoxic chemotherapy [23,24]. Blocking the tyrosine kinase activity of the endothelial cell VEGF receptor type 2 (VEGFR-2 or FLK-1) is another approach that has been considered and, currently, a number of specific inhibitors are being tested in clinical phase I–III studies. Although these inhibitors thus far have showed good tolerability, either as single agents or in combination with various cytotoxic agents, they share the theoretical disadvantage of inhibiting only angiogenic activity that is being stimulated by VEGF, and not by other pro-angiogenic stimuli. As all actively proliferating endothelial cells do express integrins, specifically blocking these integrins could have the advantage of preventing the formation of new capillaries, irrespective of the pro-angiogenic factor(s) involved. Whether this has clinical relevance, and/or whether the concurrent administration of more than one inhibitor of angiogenesis will yield superior activity, remains to be seen.

In conclusion, this phase I and pharmacokinetic study with continuous twice-weekly Cilengitide has demonstrated excellent clinical safety, even following prolonged treatment, and a predictable pharmacokinetic profile. Further studies should explore anticancer activity of Cilengitide, either as a single agent or in combination with cytotoxic agents, using different doses in a randomised study design.

References

1. Cherish D. Human endothelial cells synthesize and express an Arg-Gly-Asp directed adhesion receptor involved in attachment to fibrinogen and Van Willebrand factor. *Proc Natl Acad Sci USA* 1987, **84**, 6471–6475.
2. Cheresh D. Integrins: structure, function and biological properties. *Adv Mol Cell Biol* 1993, **6**, 225–252.
3. Varner JA, Brooks PC, Cheresh DA. Review: the integrin αvβ3: angiogenesis and apoptosis. *Cell Adhes Comm* 1995, **3**, 367–374.
4. Max R, Gerritsen RRCM, Nooijen PTGA, *et al*. Immunohistochemical analysis of integrin αvβ3 expression on tumour-associated vessels of human carcinomas. *Int J Cancer* 1997, **71**, 320–324.
5. Brooks PC, Clarke RAF, Cheresh DA. Requirement of vascular integrin αvβ3 for angiogenesis. *Science* 1994, **264**, 569–571.
6. Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. Definition of two angiogenic pathways by distinct αv integrins. *Science* 1995, **270**, 1500–1502.
7. Li X, Chen B, Blystone SD, McHugh KP, Ross FP, Ramos DM. Differential expression of αv integrins in K1735 melanoma cells. *Invasion Metastasis* 1998, **18**, 1–14.
8. Friedlander M, Theesfeld CL, Sugita M, *et al*. Involvement of integrins αvβ3 and αvβ5 in ocular neovascular diseases. *Proc Natl Acad Sci USA* 1996, **93**, 9764–9769.
9. Gasparini G, Brooks PC, Biganzoli E, *et al*. Vascular integrin αvβ3: a new prognostic indicator in breast cancer. *Clin Cancer Res* 1998, **4**, 2625–2634.
10. National Cancer Institute. *Guidelines for Reporting of Adverse Drug Reactions*. Bethesda, Division of cancer treatment, National Cancer Institute, 1988.
11. World Health Organization. *WHO Handbook for Reporting Results of Cancer Treatment*. *Offset Publication No. 40*. Geneva, Switzerland, WHO, 1979.
12. Gough K, Hutchison M, Keene O. Assessment of dose proportionality: report from the statisticians in the pharmaceutical industry/Pharmacokinetics UK joint working party. *Drug Inform J* 1995, **29**, 1039–1048.
13. Peters KG, Coogan A, Berry D, *et al*. Expression of Tie2/Tek in breast tumour vasculature provides a new marker for evaluation of tumour angiogenesis. *Br J Cancer* 1998, **77**, 51–56.
14. Hayes AJ, Huang WQ, Mallah J, Yang D, Lippman ME, Li LY. Angiopoeitin-1 and its receptor Tie-2 participate in the regulation of capillary-like tube formation and survival of endothelial cells. *Microvasc Res* 1999, **58**, 224–237.
15. Hebbar M, Revillion F, Louchez MM, Fournier C, Bonneterre J, Peyrat JP. Prognostic value of circulating soluble E-selectin concentrations in node-negative breast cancer patients. *Clin Cancer Res* 1999, **5**, 1427–1433.
16. Wynendaele W, Derua R, Hoylaerts MF, *et al*. Vascular endothelial growth factor measured in platelet poor plasma allows optimal separation between cancer patients and volunteers: a key to study an angiogenic marker in vivo? *Ann Oncol* 1999, **10**, 965–971.
17. Barleon B, Siemeister G, Martiny-Baron G, Weindel K, Herzog C, Marme D. Vascular endothelial growth factor up-regulates its receptor fms-like tyrosine kinase (FLT-1) and a soluble form of FLT-1 in human vascular endothelial cells. *Cancer Res* 1997, **57**, 5421–5425.
18. Cutler NR, Sramek JJ, Greenblatt DJ, Chaikin P, Collins J. Defining the maximum tolerated dose: an update. *J Clin Pharmacol* 2000, **40**, 1183–1204.
19. Arbuck SG. Workshop on phase I study design. Ninth NCI/EORTC New Drug Development Symposium, Amsterdam, March 12, 1996. *Ann Oncol* 1996, **7**, 567–573.
20. Gutheil JC, Campbell TN, Pierce PR, *et al*. Targeted antiangiogenic therapy for cancer using Vitaxin: a humanized monoclonal antibody to the integrin αvβ3. *Clin Cancer Res* 2000, **6**, 3056–3061.
21. Eskens FALM, Verweij J. Clinical studies in the development of new anticancer agents exhibiting growth inhibition in models: facing the challenge of a proper study design. *Crit Rev Oncol Hematol* 2000, **34**, 83–88.
22. Gelmon KA, Eisenhauer EA, Harris AL, Ratain MJ, Workman P. Anticancer agents targeting signaling molecules and cancer cell environment: challenges for drug development? *J Natl Cancer Inst* 1999, **91**, 1281–1287.
23. Gordon MS, Margolin K, Talpaz M, *et al*. Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. *J Clin Oncol* 2001, **19**, 843–850.
24. Margolin K, Gordon MS, Holmgren E, *et al*. Phase Ib trial of recombinant humanized monoclonal antibody to vascular endothelial growth factor in combination with chemotherapy in patients with advanced cancer: pharmacologic and long-term safety data. *J Clin Oncol* 2001, **19**, 851–856.