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Phase I clinical and pharmacokinetic study of PTK/ZK, a multiple VEGF receptor inhibitor, in patients with liver metastases from solid tumours

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Abstract

The family of VEGF receptors are important mediators of angiogenesis, which is essential for tumour growth and metastasis. PTK/ZK is a multiple VEGF receptor inhibitor that blocks the activity of all known VEGF receptor tyrosine kinases. This phase I/II trial evaluated the safety, pharmacokinetics and efficacy of PTK/ZK in patients with liver metastases from solid tumours. Patients were administered oral PTK/ZK monotherapy once daily at doses of 300–1200 mg/day in 28-day cycles until unacceptable toxicity or tumour progression occurred. Twenty-seven patients were enrolled and treatment with PTK/ZK was generally well tolerated. The most frequently reported adverse events were fatigue, nausea, dizziness, and vomiting (mostly National Cancer Institute Common Toxicity Criteria grade 1 or 2). The area under the concentration–time curve (AUC) of PTK/ZK increased between 300 and 1000 mg/day with no further increase from 1000 to 1200 mg/day; the AUC decreased by 50% between day 1 and day 15. The DCE-MRI showed a statistically significant early reduction of tumour blood supply (measured as Ki) at day 2 at doses $\geqslant$750 mg/day. Disease progression was significantly correlated with percent change from baseline Ki. Thirteen patients had stable disease for at least two cycles (56 days). Median overall survival was 11.8 months (95% CI = 6.6, 17.1 months). Long-term therapy with PTK/ZK demonstrated predictable pharmacokinetics, was safe and feasible in patients with metastatic disease, and showed promising clinical activity. The minimum biologically active dose was established at 750 mg/day whereas the recommended dose for phase III studies is 1200 mg/day.

Keywords: Vascular endothelial growth factor; Antiangiogenesis; Tyrosine kinase inhibitor; Colorectal; Vatalanib

1. Introduction

As first recognised by Folkman in 1971 [1], angiogenesis is fundamental to tumour growth and metastasis. Angiogenesis is implicated in the initial progression from a premalignant tumour to a cancer [2], haematological dissemination [3] and in the growth of dormant micrometastases into overt metastatic lesions [4]. In recent years, several drugs designed to inhibit angiogenesis and thus tumour growth have entered clinical trials with the role of antiangiogenic therapy as cancer treatment under extensive review [5–7].

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Of the numerous growth factors and cytokines shown to have angiogenic activity, the VEGF family of ligands (VEGF-A, -B, -C, -D, and -E), which act through the VEGF family of receptors (VEGFR-1, -2, and -3) [8,9], are key mediators of tumour-induced neovascularisation and enhancement of vascular permeability [10,11]. The VEGF receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR), which are predominantly located on proliferating endothelial cells, are upregulated during neovascularisation. Binding of any of five VEGF ligands to any of the three VEGF receptors activates the receptor tyrosine kinase, triggering signaling pathways that stimulate endothelial cell proliferation, migration and tube formation. PTK/ZK is a multiple VEGF receptor inhibitor that blocks the intracellular tyrosine kinase activity of all known VEGF receptors and is therefore suitable for long-term therapy of pathologic tumour neovascularisation [12]. In several preclinical models, PTK/ZK caused a significant decrease in vessel permeability, an increase in tumour blood volume and a reduction in tumour vessel density [12,13]. In the current report, we describe the first clinical, pharmacokinetic (PK) and correlative laboratory findings from a phase I study of PTK/ZK monotherapy in patients with liver metastasis secondary to solid tumours.

2. Patients and methods

2.1. Patients

Patients with breast or colorectal carcinoma and liver metastasis for which no standard curative therapy was available were eligible for this study. The liver metastasis was evaluated for tumour size and blood flow by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and Doppler ultrasound examination. Other inclusion criteria were WHO performance score $\leqslant 2$, an estimated life expectancy $\geqslant 3$ months and adequate bone marrow function, renal function and liver function defined as absolute neutrophil count (ANC) 1500/μL, platelet count $\geqslant 100\,000$ μL, haemoglobin $\geqslant 9$ g/dL, serum creatinine $\leqslant 1.5 \times$ upper limit of normal (ULN), serum total bilirubin $\leqslant 1.5 \times$ ULN, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) $\leqslant 2.5 \times$ ULN, 24-h creatinine clearance $\geqslant 50$ mL/min and urinary protein excretion <0.5 g/24 h. All patients provided a written informed consent. Prior therapies were permitted if administered $\geqslant 6$ weeks before study entry for nitrosoureas or mitomycin C, $\geqslant 2$ weeks before study entry for radiotherapy or surgical procedure or $\geqslant 4$ weeks before study entry for any other cytotoxic, cytostatic or investigational drug. Patients were not included if they were pregnant or breast feeding, had a history of brain metastasis or high-dose chemotherapy with haematologic stem cell support. Other exclusion criteria were concurrent severe and/or uncontrolled medical disease that could compromise participation in the study.

2.2. Treatment and study design

Patients were treated with oral PTK/ZK monotherapy at doses ranging from 300 to 1200 mg/day. The dose was taken once per day for a 28-day treatment cycle. A standard phase I study design was used wherein three to six patients were enrolled per dose cohort (modified Fibonacci series). Dose escalation occurred when three to six patients had been treated for at least 4 weeks with 0/3 or 1/6 patients experiencing dose-limiting toxicity (DLT). The dose was escalated up to 1200 mg/day.

An adverse event (AE) or laboratory abnormality was considered to be a DLT if it occurred during the first 28 days following the first dose of PTK/ZK (cycle 1) and if it met any of the following criteria: CTC grade 4 neutropenia (ANC, including bands, $<0.5 \times 10$ 9/L); grade 4 anemia (Hgb <6.5 g/dL); grade 4 thrombocytopenia (platelet count $<10.0 \times 10$ 9/L); grade 3 or 4 non-heamatologic adverse events; $\geqslant$grade 2 proteinuria; $\geqslant$grade 2 hematuria; serum creatinine $\geqslant 2.0 \times$ ULN; certain grade 2 toxicities (*e.g.*, neurotoxicity or cardiac toxicity) may have been considered dose limiting following consultation between the investigator and Schering AG. The following were not considered to be DLTs: grade 3 nausea or grade 3 or 4 vomiting (grade 3 or 4 vomiting was only considered dose limiting if it occurred despite the use of standard antiemetics); grade 3 or 4 fever identified with a source (*i.e.*, infection, tumour); grade 3 or 4 alkaline phosphatase.

The duration of each dosing cycle was defined as 28 days. Maximum tolerated dose (MTD) was defined as the dose level at which at most 1/6 patients experienced a DLT, with at least two patients experiencing DLT at the next higher dose level.

Tumour response was assessed at the end of each treatment cycle according to Southwest Oncology Group (SWOG) criteria [14]. The study protocol and all amendments were fully reviewed and approved by the ethical committee of the University of Freiburg.

2.3. Pharmacokinetic sample collection and analysis

Blood samples (3 mL) were obtained immediately before treatment (predose) and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 10, and 24 h (before next dose) on days 1, 15, and 28 during the first treatment cycle. Plasma was then frozen at −80 °C until analysis. The concentration of PTK/ZK and 4 metabolites in plasma was measured using a validated reversed-phase high pressure liquid chromatography (HPLC) system under isocratic conditions with ultraviolet detection by AAI, Germany. The sample preparation involved a liquid–liquid extraction by

t-butylmethylether after adding an internal standard. The method was linear over the calibration range (2.5–10 000 ng/mL).

The following PK parameters were calculated for plasma concentration: the apparent volume of distribution during terminal phase [Vz/F], apparent plasma clearance (CL/F), area under the curve (AUC), terminal elimination half-life ($t_{1/2}$), maximum concentration (C_{max}), and time to maximal concentration (t_{max}) were determined by noncompartmental analysis. The program WinNonlin Version 4.0 Professional (Pharsight®, Mountain View, Calif, USA) was used for PK analysis.

2.4. Surrogate biomarker sample collection

Blood samples for the measurement of VEGF-A, bFGF, sFlt1, sTie-2, and sE-selectin were taken at baseline, at day 1 predose and 10 h after drug intake; predose at days 8, 15, 22, and 28 in the first cycle; and predose on days 15 and 28 during subsequent treatment cycles. Plasma VEGF-A and bFGF, and serum sFlt-1, sTie-2, and sE-selectin were measured by ELISA (R&D Systems Europe, Oxford, UK).

2.5. Dynamic contrast-enhanced magnetic resonance imaging

The DCE-MRI studies were performed according to a protocol recently published in detail [15]. Tumour permeability and vascularity were assessed by calculating the bidirectional transfer constant (Ki). Patients were imaged at baseline and between 2 and 4 h postdose on day 2 and postdose on day 28 of each treatment cycle. Enhancement parameters for Ki were expressed as a percentage of the baseline value. The degree of association between percent of baseline Ki, dose, and drug plasma exposure (AUC, C_{max}, and C_{min}) was assessed by the Spearman Rank correlation coefficient. Mean percent of baseline Ki and 95% confidence intervals (CI) by timepoint for patients with progressive disease *versus* patients with no progressive disease were calculated and compared using the Mann–Whitney *U* test.

2.6. Colour doppler imaging (CDI) for blood flow measurements in tumour vessels

Blood flow measurements in tumour vessels were performed using Doppler ultrasound flow imaging. All measurements were performed with a Logic 700 (General Electric, Ultrasound Europe, Dachau, Germany) and 90% of all measurements was performed by one experienced person. Assessments were performed at baseline, predose and 2–4 h postdose on day 3 of cycle 1, and postdose on day 28 of each treatment cycle. The systolic and diastolic blood flow was measured in the tumour vessel and in the hepatic artery. Heart rate and blood pressure were measured. Because of imaging limitations, the tumour lesions measured by CDI were not always identical to those imaged in the DCE-MRI studies. The resistance index (RI) was used for calculations/correlations and was determined by the formula: $RI = (V_{sys} - V_{dia})/V_{sys.}$. The difference between the RI at day 0 and day 28 (Δ) was determined for the tumour vessel and for the hepatic artery, a $-\Delta$ would mean a lower blood flow and a $+\Delta$ would mean a higher blood flow.

3. Results

3.1. Patients

A total of 27 patients were enrolled onto this trial and were treated until disease progression or unacceptable toxicity occurred, with the exception of 7 patients who discontinued therapy with PTK/ZK as a result of a temporary clinical hold due to unexpected preclinical findings. The trial was resumed after it was established that similar effects did not occur in clinical trials. Patient demographics and baseline disease characteristics are shown in Table 1. Approximately 85% of patients had received three or more prior regimens of chemotherapy and 67% of patients had two or more metastatic organs involved at baseline. All patients but one had confirmed liver metastasis secondary to either colorectal cancer ($n = 23$) or breast cancer ($n = 3$). Three patients were treated at each of the following dose levels: 300, 500, 750, and 1000 mg/day; 15 patients were treated at 1200 mg/day. The median duration of exposure to PTK/ZK for all patients was 58 days (range 3–310 days).

3.2. Toxicity and tolerability

All patients were evaluable for safety. The most frequently reported adverse events (AEs) experienced by $\geqslant 20\%$ of patients regardless of study drug relationship are shown in Table 2. These were predominantly National Cancer Institute Common Toxicity Criteria (CTC) grade 1 or 2 in severity and consisted primarily of fatigue, nausea, dizziness, and vomiting. The incidence of Grade 3 and 4 toxicities are reported in Table 3. Serious adverse events suspected to be related to study drug occurred in three patients: one with grade 3 ataxia at 1200 mg/day; one with grade 3 dyspnea and hypertension at 1200 mg/day and one with grade 4 hypertensive crisis at 500 mg/day, which fell outside of the 28 determination period for dose-limiting toxicity (DLT). With the exception of persistent grade 3 hypertension in one patient, all of these clinically manageable events were reversed without incident upon discontinuation of PTK/ZK.

Table 1
Patient demographics and baseline disease characteristics

Characteristic	Patients, *n* (%)				
	<750 mg	750–1000 mg	1200 mg (dose escalation)	1200 mg (dose expansion)[a]	Total
n	6	6	9	6	27
Sex					
Male	3 (50.0)	4 (66.7)	3 (33.3)	5 (83.3)	15 (55.6)
Female	3 (50.0)	2 (33.3)	6 (66.7)	1 (16.7)	12 (44.4)
Race					
Caucasian	6 (100)	6 (100)	9 (100)	6 (100)	27 (100)
Performance status					
0–1	6 (100)	6 (100)	8 (88.9)	6 (100)	26 (96.3)
⩾2	0 (0)	0 (0)	1 (11.1)	0 (0)	1 (3.7)
Age, years					
Median	60.5	59.0	53.0	57.0	58.0
Range	38–74	54–71	41–67	45–67	38–74
Primary tumour type					
Breast	1 (16.7)	0 (0)	2 (22.2)	0 (0)	3 (11.1)
Colorectal	5 (83.3)	6 (100.0)	6 (66.7)	6 (100.0)	23 (85.2)
Leiomyosarcoma	0 (0)	0 (0)	1 (11.1)	0 (0)	1 (3.7)
Prior antineoplastic therapies, n					
1	1 (16.7)	1 (16.7)	0 (0.0)	0 (0.0)	2 (7.4)
2	0 (0.0)	0 (0.0)	1 (11.1)	1 (16.7)	2 (7.4)
3	2 (33.3)	2 (33.3)	3 (33.3)	0 (0.0)	7 (25.9)
⩾4	3 (50.0)	3 (50.0)	5 (55.6)	5 (83.3)	16 (59.3)
Organs involved, *n*					
1	1 (16.7)	4 (66.7)	3 (33.3)	1 (16.7)	9 (33.3)
2	1 (16.7)	1 (16.7)	4 (44.4)	3 (50.0)	9 (33.3)
⩾2	4 (66.7)	1 (16.7)	2 (22.2)	2 (33.3)	9 (33.3)

[a] Dose expansion patients are reported separately because, per protocol, these patients were not evaluable for establishing dose-limiting toxicity.

Absolute lymphocytes was the most common newly-occurring or worsening grade 3 haematological abnormality ($n = 5$ patients) without any clinical symptom associated with this haematological abnormality. One patient experienced grade 4 neutropenia at a dose level of 750 mg/day. Alkaline phosphatase and serum glutamate pyruvate transaminase (SGPT) ($n = 4$ patients each) were the most common newly-occurring or worsening grade 3 blood chemistry abnormality reported. No patients experienced grade 4 biochemistry abnormalities. Changes in haematology and biochemistry did not appear to be dose-related.

Two patients at 1200 mg/day had DLTs. One patient with a history of migraine headaches had DLTs of grade 3 ataxia and grade 3 vertigo, and one patient with a history of diabetes mellitus and arterial hypertension had DLTs of hypertension, ataxia and dizziness, all grade 3. No patients in the other cohorts had DLTs. Maximum tolerated dose (MTD) in this study population was thus determined to be 1000 mg/day. However, safety, PK, and MRI data from this and a second, ongoing phase I trial with PTK/ZK given once daily, where 15 patients cleared dosages ⩾1000 mg/day without establishing the MTD dose level, suggested that the overall best risk/benefit strategy was to expand accrual at 1200 mg/day to further evaluate safety and tolerability of dose and to provide increased activity. From a safety perspective, it is important to note that all observed DLTs were completely reversible following interruption of PTK/ZK treatment in this trial. There was only one instance of a patient experiencing protracted side effect symptoms upon drug discontinuation, where persistent grade 3 hypertension was observed, however, the patient's own history of hypertension likely influenced the toxicity. Therefore six additional patients were enrolled at 1200 mg/day for further analysis of pharmacokinetics, efficacy, and overall safety.

Adverse events which led to premature discontinuation from the trial ($n = 5$ patients) were hypertension, vertigo, ataxia, dyspnoea, nausea, and vomiting. The most common grade 3 adverse events that occurred in more than one patient, regardless of study drug relationship, were hypertension ($n = 5$), ataxia ($n = 3$), dizziness ($n = 2$), and dyspnoea ($n = 2$). There were no grade 4 adverse events that occurred in more than one patient, regardless of study drug relationship. The incidence of these adverse events was not dose-related. In several pa-

Table 2
Adverse events occurring in ⩾20% of patients regardless of study drug relationship

Preferred term	Grade	Patients, *n* (%)			
		<750 mg (*n* = 6)	750–1000 mg (*n* = 6)	1200 mg (*n* = 15)	Total (*n* = 27)
Fatigue	1	2 (33)	3 (50)	7 (47)	12 (44)
	2	0	2 (33)	5 (33)	7 (26)
Nausea	1	5 (83)	4 (67)	6 (40)	15 (56)
	2	0	1 (17)	3 (20)	4 (15)
Dizziness	1	1 (17)	4 (67)	7 (47)	12 (44)
	2	0	0	2 (13)	2 (7)
	3	0	1 (17)	1 (7)	2 (7)
Vomiting	1	2 (33)	1 (17)	5 (33)	8 (30)
	2	0	1 (17)	4 (27)	5 (19)
	4	1 (17)	0	0	1 (4)
Diarrhoea	1	1 (17)	1 (17)	4 (27)	6 (22)
	2	0	0	1 (7)	1 (4)
Sweating increased	1	0	1 (17)	5 (33)	6 (22)
	2	0	0	1 (7)	1 (4)
Vertigo	1	2 (33)	2 (33)	1 (7)	5 (19)
	2	0	0	1 (7)	1 (4)
	3	0	0	1 (7)	1 (4)
Ataxia	1	0	1 (17)	1 (7)	2 (7)
	2	0	0	1 (7)	1 (4)
	3	0	1 (17)	2 (13)	3 (11)
Dyspnoea	1	0	1 (17)	2 (13)	3 (11)
	2	0	0	1 (7)	1 (4)
	3	1 (17)	0	1 (7)	2 (7)
Hypertension	2	1 (17)	0	0	1 (4)
	3	1 (17)	1 (17)	3 (20)	5 (19)

tients the observed toxicity considered to be related to PTK/ZK occurred at the beginning of treatment and decreased over time, corresponding to the decrease in AUC from day 1 to day 15.

3.3. Pharmacokinetics of PTK/ZK

Plasma concentrations *versus* time profile of PTK/ZK after a single dose of 300–1200 mg/day are shown in Fig. 1. Peak (C_{max}) and total systemic exposure (AUC) to PTK/ZK increased with dose after both single and multiple doses of PTK/ZK between 300 and 1000 mg/day, with no further increase between 1000 and 1200 mg/day (Fig. 2). Mean plasma concentrations over time for patients administered 1200 mg/day are shown for days 1, 15, and 28 in Fig. 3. Exposure to PTK/ZK decreased by approximately 50% between day 1 and day 15 of treatment, with no further declines observed between day 15 and day 28 of treatment. The decrease in systemic exposure after multiple doses of PTK/ZK could be explained by auto-induction in drug metabolism, as evidenced by an increase in apparent clearance between day 1 and day 15 of treatment. The mean pharmacokinetic parameters at 1200 mg/day are shown in Table 4. Four metabolites of PTK/ZK have been identified in plasma (data not shown); however, PTK/ZK was the major pharmacologically active moiety observed in plasma after single and multiple doses.

3.4. Clinical efficacy

Of 27 patients enrolled, 21 were assessable for best tumour response by SWOG criteria. Of the remaining 6 patients, best response was inevaluable because of premature discontinuation of these patients. Although no complete or partial responses were observed, 13 (48%) patients had stable disease for at least two cycles (56 days). Median overall survival was 11.8 months (95% CI = 6.6, 17.1 months).

Table 3
Grade ⩾3 toxicities for all dose levels

Preferred term	Grade	Patients, *n* (%)			
		<750 mg (*n* = 6)	750–1000 mg (*n* = 6)	1200 mg (*n* = 15)	Total (*n* = 27)
Urinary tract infection	3	0	0	1 (7)	1 (4)
Bile duct obstruction	3	0	0	1 (7)	1 (4)
Hyperlipidaemia	3	0	0	1 (7)	1 (4)
Hyperbilirubinaemia	3	0	0	1 (7)	1 (4)
Abdominal pain upper	3	0	1 (17)	0	1 (4)
Neutropenia	4	0	1 (17)	0	1 (4)
Dizziness	3	0	1 (17)	1 (7)	2 (7)
Diarrhoea	3	0	1 (17)	0	1 (4)
Vomiting	4	1 (17)	0	0	1 (4)
Paraesthesia	3	0	1 (17)	0	1 (4)
Cerebral ischemia	3	0	0	1 (17)	1 (4)
Hoarseness	3	0	0	1 (7)	1 (4)
Dyspnoea	3	1 (17)	0	1 (17)	2 (7)
Dyspnoea exacerbated	4	1 (17)	0	0	1(4)
Phlebothrombosis	4	0	0	1 (7)	1 (4)
Vertigo	3	0	0	1 (7)	1 (4)
Ataxia	3	0	1 (17)	2 (13)	3 (11)
Hypertension	3	1 (17)	1 (17)	3 (20)	5 (19)
Hypertensive crisis	4	1 (17)	0	0	1 (4)

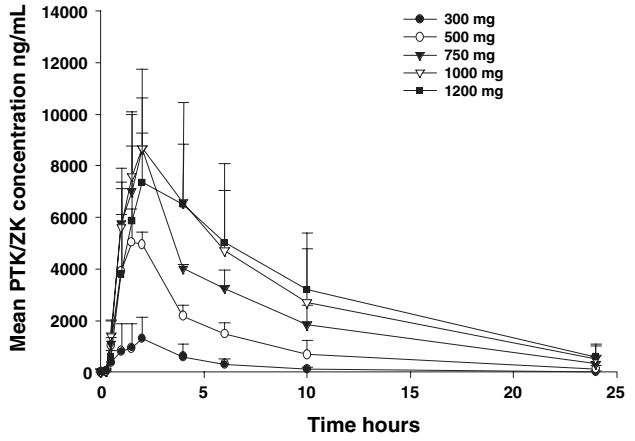

Fig. 1. Mean plasma concentration over time for each dose level of PTK/ZK on day 1.

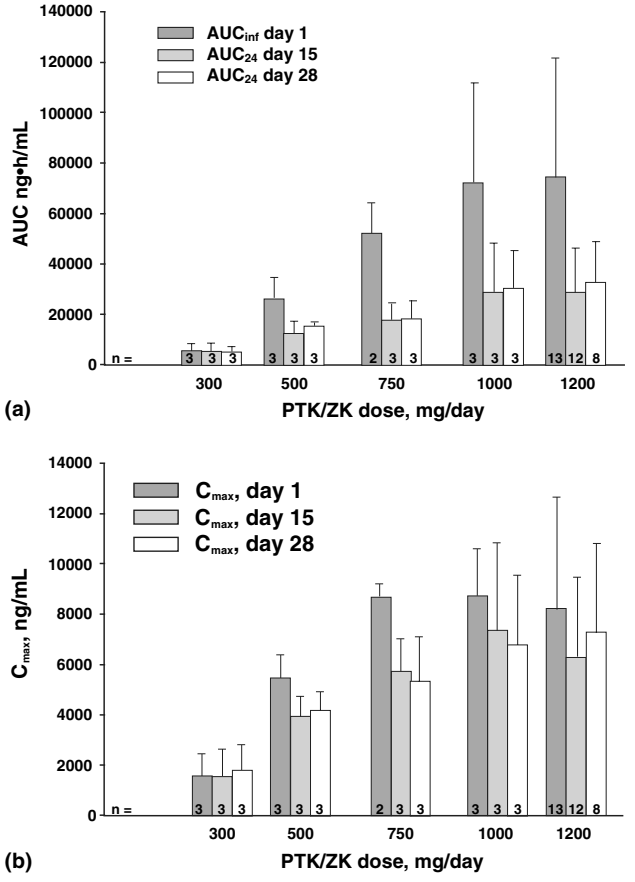

Fig. 2. (a) Area under the concentration–time curve, and (b) maximum plasma concentrations for PTK/ZK by dose level and day of administration.

3.5. Surrogate biomarkers of angiogenesis

The effects of PTK/ZK administration on 5 different plasma/serum biomarkers of angiogenesis (VEGF-A, bFGF, sFlt1, sTie2, and sE-selectin) were evaluated in this trial. These results have been published in detail elsewhere [16]. In this trial, plasma VEGF-A and bFGF increased postdose of PTK/ZK, while no changes were seen in the 3 serum biomarkers after dosing; however, none of the plasma/serum biomarkers evaluated showed any correlation with disease progression.

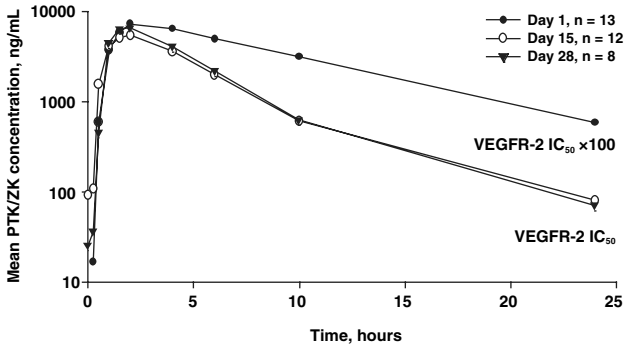

Fig. 3. Mean plasma concentration over time at days 1, 15, and 28 for patients treated with oral PTK/ZK 1200 mg/day.

3.6. Colour doppler imaging blood flow measurements

Colour Doppler Imaging (CDI) was performed on all patients evaluable for response. Despite a small increase from baseline in tumour blood flow over the first cycle of treatment, this parameter was not predictive of disease progression.

3.7. Dynamic contrast-enhanced magnetic resonance imaging

Comparative MRI studies of liver metastases were performed before treatment, after treatment on day2, and after the end of each 28-day treatment cycle in all patients evaluable for response. The reduction of tumour blood supply, as indicated by percent of baseline Ki, by dose of PTK/ZK on day 2, end of cycle 1 (day 28), and end of cycle 2 (day 56) is shown in Fig. 4. Tumour blood supply over the first cycle of treatment was significantly and inversely correlated with systemic exposure of PTK/ZK, including dose, C_{min}, C_{max}, and AUC (Table 5). The relationship between change from baseline Ki, AUC, and C_{min} on day 2 and end of cycle 1 was best described by an inhibitory E_{max} model similar to previously published data in patients with colorectal cancer [15]. A dose of 750 mg/day was likely to significantly decrease tumour blood supply (Ki) on day 2 but not at the end of cycle 1, whereas doses $\geqslant$1000 mg/day were more likely to significantly decrease tumour blood supply on both day 2 and end of cycle 1 (Table 6). This suggests that the minimum biologically active dose for patients with colorectal cancer could be as low as 750 mg/day, but that the optimal biologic dose would be $\geqslant$1000 mg/day.

Table 4
Mean pharmacokinetic parameters of oral PTK/ZK with once-daily dosing at 1200 mg/day

PK parameter	Mean ± SD (%CV)		
	Day 1 (n = 13)	Day 15 (n = 12)	Day 28 (n = 8)
t_{max} (h)	2.6 ± 1.0 (39)	1.9 ± 1.0 (50)	2.1 ± 0.9 (42)
C_{max}, ng/mL (μM)	8180 ± 4520 (55) (23.6)	6230 ± 3290 (53) (18)	7300 ± 3500 (49) (21)
AUC_{0-24}, ng/h/mL (μM/h)	69 200 ± 43 400 (63) (200)	28 800 ± 17 600 (61) (83)	32 500 ± 16 300 (50) (93)
Elimination $t_{1/2}$ (h)	5.3 ± 1.6 (30)	3.5 ± 1.0 (29)	4.0 ± 0.7 (18)
CL/F (L/h)	37 ± 45 (124)	67.2 ± 57.9 (86)	50.1 ± 33.2 (66)
Vz/F (L)	235 ± 252 (107)	306 ± 251 (82)	316 ± 270 (86)

PK, pharmacokinetics; SD, standard deviation; CV, coefficient of variation; t_{max}, time to reach C_{max}; C_{max}, maximum serum concentration; AUC_{0-24}, area under the concentration–time curve from 0 to 24 h; $t_{1/2}$, half-life; CL/F, Apparent oral clearance; Vz/F, apparent volume of distribution during terminal phase.

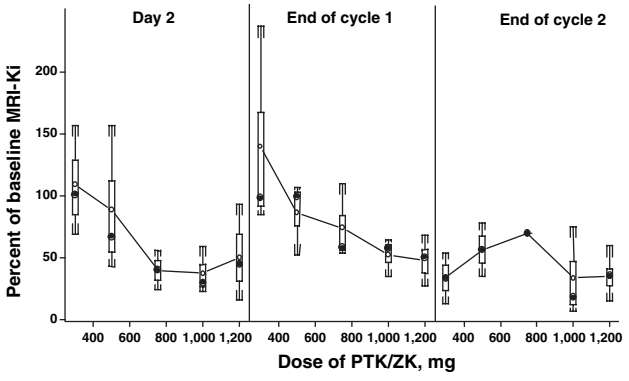

Fig. 4. Correlation between percent change from baseline Ki and dose of PTK/ZK on days 2, 28, and 56 in patients with solid tumours treated with oral, once-daily PTK/ZK monotherapy.

Table 5
Correlation between dose of PTK/ZK, percent change from baseline Ki, and systemic exposure

	Day 2		End of cycle 1	
	Coefficient	P value	Coefficient	P value
Dose (mg)	−0.445	0.048	−0.654	0.0024
AUC	−0.827	<0.0001	−0.571	0.011
C_{max}	−0.736	0.0005	−0.468	0.0431
C_{min}	−0.87	<0.0001	−0.499	0.0351

AUC, area under the concentration–time curve; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration.

Moreover, tumour blood supply (percent of baseline Ki) on day 2 and end of cycle 1 was significantly correlated with clinical outcome (stable *versus* progressive disease; Table 7). The best clinical response observed over the first 2 cycles of treatment was significantly predicted by changes in percent of baseline Ki observed on days 2 and 28. Patients with a best clinical response of stable disease had a consistently greater reduction in Ki compared to patients with disease progression on day 2 (49.3% *versus* 90.3%; $P = 0.038$) and at the end of cycle 1 (56.4% *versus* 101.4%; $P = 0.039$). In the subset of patients with colorectal cancer, the reduction in Ki among patients with stable *versus* progressive disease was not statistically significant at day 2 (49.3% *versus* 80.2%; $P = 0.14$) but was statistically significant at the end of cycle 1 (56.4% *versus* 111.6%; $P = 0.027$).

Table 6
ANOVA results of change in Ki *versus* dose of PTK/ZK

Dose (mg)	Mean effect		P-value	
	Day 2	End of cycle 1	Day 2	End of Cycle 1
Overall	–	–	0.087	0.035
300	8.84	39.72	0.672	0.088
500	−11.39	−13.91	0.586	0.531
750	−60.22	−26.21	0.031	0.247
1000	−62.87	−48.04	0.008	0.044
1200	−49.00	−52.56	0.001	0.002
Combined low-and high-dose analyses				
⩽750 mg	−16.01	−0.14	0.242	0.992
⩾1000 mg	−53.51	−51.20	0.0002	0.0008

ANOVA, analysis of variance; Ki, bidirectional transfer constant, which approximates tumour blood flow.

4. Discussion

This study was primarily designed to assess the safety and pharmacokinetic profiles of PTK/ZK in patients with liver metastasis and to establish a recommended best effective dose for phase II and III trials. For this reason, analyses of tumour blood supply and serum biomarkers of angiogenic activity were incorporated into the study protocol to provide further clinical guidance for recommendation of a selected dose for phase II and III trials. We have demonstrated that PTK/ZK, when administered to a group of heavily pretreated patients with liver metastases, is safe and well tolerated as long-term therapy at doses ranging from 300 to 1200 mg/day. The most frequently reported adverse events were fatigue, nausea, dizziness, and vomiting,

Table 7
Correlation between percent change from baseline Ki and best clinical outcome after 2 treatment cycles

	All patients (%)				Colorectal patients (%)			
	PD	SD	Δ	*P* value	PD	SD	Δ	*P* value
Day 2	90.4	49.3	41.2	0.038	80.2	49.3	30.9	0.14
End of cycle 1	101.4	56.4	45	0.039	111.6	56.4	55.2	0.027

Ki, bidirectional transfer constant; PD, progressive disease; Δ, difference between mean change in Ki for patients with PD *versus* SD; SD, stable disease.

mostly of CTC grade 1 and 2. A total of 5 patients discontinued the study prematurely due to adverse events. Although no partial responses or complete responses were observed, 13 (48%) patients experienced stable disease for $\geqslant$56 days. Median overall survival was 11.8 months (95% CI = 6.6, 17.1 months). This data compares favourably to a previous report of patients with metastatic colorectal cancer treated with second-line fluorouracil; that study reported a median survival of 8.5 months [17].

PTK/ZK is rapidly absorbed after oral administration with a t_{max} observed at 1.0–1.5 h postdose, then eliminated with a mean terminal half-life of 3.5–5 h. The rate (C_{max}) and extent (AUC) of exposure increased with dose up to 1000 mg/day, then saturated with no further increase observed at 1200 mg/day. Upon multiple dosing, the AUC declined by 50% between day 1 and day 15 of treatment and remained at steady-state thereafter. This decline in AUC between day 1 and day 15 is consistent with auto-induction in clearance. Corresponding to this reduction of drug exposure by time at the same dose level, there was a reduction of adverse symptoms over time as the more rapid drug clearance occurred.

Some studies have suggested that circulating levels of VEGF ligands may be a useful prognostic marker. However, results were not consistent and were limited by the retrospective nature of most studies [18]. In our study, serum/plasma assays of the angiogenesis biomarkers tested demonstrated substantial interpatient heterogenicity, with no consistent change during therapy with PTK/ZK. It is possible, therefore, that measuring upstream signals that promote angiogenesis *via* receptors blocked by PTK/ZK has limited value as an indicator of antiangiogenic effect.

This study also examined the use of two exploratory imaging techniques, DCE-MRI and CDI, as surrogate markers of clinical activity. Despite a small increase in tumour blood flow as measured by Doppler ultrasound, there were no significant differences between patients with stable *versus* progressive disease, possibly because of technical limitations associated with this technique. Successful assessment *via* CDI requires a high level of technical skill to achieve reproducible results with repeated imaging, thus limiting the broad utility of this technique. Furthermore, other factors can influence tumour microvasculature, including interstitial fluid pressure, blood pressure, blood viscosity, and drugs that induce vasodilation or vasoconstriction, as discussed in detail elsewhere [19]. These factors probably are not constant and cannot be controlled within a clinical study.

In contrast, DCE-MRI, as an indicator of tumour vascularity and/or permeability, was a reliable method of evaluating tumour blood supply, as confirmed by previous publications [13,15,20]. Our DCE-MRI studies demonstrated a dose-dependent and significant decrease in Ki from baseline following administration of PTK/ZK. The Ki reflects tumour vascularity and/or permeability determined by the disposition of the contrast-enhancing agent (*i.e.*, gadolinium), the distribution of gadolinium from the tumour vessel into the tumour interstitium, and the redistribution back into the central compartment. Interestingly, patients with stable disease had significantly reduced Ki from baseline to a much greater extent than in patients with progressive disease. This early PTK/ZK-induced effect was noticeable as early as day 2 of treatment and persisted at the end of cycle 1, and was significantly correlated with clinical outcome after 2 months of continuous therapy with PTK/ZK. A more detailed presentation of these findings that also incorporated data from another phase I study with PTK/ZK was published recently [15].

Successful antiangiogenic therapy appears to require continuous administration over long periods of time and for such an approach an effective and safe oral drug is necessary. PTK/ZK downregulates neovascularisation by inhibiting the proliferation and migration of endothelial cells blockade of the VEGF receptor family signalling pathways, especially VEGFR-2 (KDR). The regression or involution of a vigorously growing capillary tumour bed is a slower process than the lysis of tumour or endothelial cells by cytotoxic agents. Therefore, traditional tumour assessments such as those of SWOG, which require changes in tumour regression or progression of at least 50%, may not be appropriate tools for assessment of clinical activity with cytostatic antiangiogenic agents.

In conclusion, our study demonstrates that continuous prolonged PTK/ZK monotherapy is feasible and tolerable in patients with liver metastasis. PTK/ZK demonstrated predictable pharmacokinetics and administra-

tion was correlated with significant reduction in blood supply in tumour metastases. These efficacy results are particularly promising in this study population of patients with advanced metastatic disease, and when compared to historical reports of studies with similar patient populations. Moreover, preliminary reports of other phase I/II trials of PTK/ZK reveal promising activity in monotherapy treatment for other types of solid tumours, including renal cell carcinoma and glioblastoma [21,22]. Based on the results from this study, the minimum biological dose for activity in patients with colorectal cancer and liver metastases is 750 mg/day, with an optimal dose between 1000 and 1200 mg/day. On the basis of the results from this and other phase I/II trials, PTK/ZK became the first of this class of angiogenesis inhibitors to enter phase III trials. Two phase III studies of PTK/ZK in combination with the oxaliplatin-containing FOLFOX regimen in patients with colorectal cancer (CONFIRM-1 and CONFIRM-2) are currently enrolling at 1250 mg/day and the results of those trials are awaited with interest.

Conflict of interest statement

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