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Phase 1 Study of Combination Treatment with PTK 787/ZK 222584 and Cetuximab for Patients with Advanced Solid Tumors: Safety, Pharmacokinetics, Pharmacodynamics Analysis[1]

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Abstract

INTRODUCTION: PTK/ZK is a small-molecule inhibitor of all three vascular endothelial growth factor (VEGF) receptors, platelet-derived growth factor receptor, colony-stimulating factor 1 receptor, and cytokine stem cell factor receptor. Cetuximab is a monoclonal antibody against epidermal growth factor (EGF) receptor. Combining inhibition of VEGF and EGF signaling might act additive or synergistically. *METHODS:* In phase 1 design, patients with advanced solid tumors were treated with PTK/ZK daily (cohort 1, 750 mg once daily; cohort 2, 1250 mg once daily; cohort 3, 250 mg [morning] and 500 mg [evening]; and cohort 4, 500 mg [morning] and 750 mg [evening]) in combination with cetuximab 250 mg/m^2 weekly in cycles of 28 days in cohorts of three patients. Toxicity was evaluated conform the Common Terminology Criteria for Adverse Events classification 3.0. Pharmacokinetics and pharmacodynamics consisting of circulating endothelial (progenitor) cell (CE[P]C) analysis by flow cytometry were performed. *RESULTS:* Safety and tolerability was evaluated in 16 patients. The most frequently reported adverse events were acne, dry skin, fatigue, nausea, dizziness, vomiting, headache, and diarrhea. One dose-limiting toxicity occurred in cohort 3 consisting of a grade 3 transaminitis. Pharmacokinetic analysis revealed no significant changes in PTK/ZK exposure on coadministration with cetuximab and in bioavailability at equivalent total daily doses. Biomarker analysis showed no significant change in the number of CE(P)Cs during treatment. One of 14 evaluable patients showed a partial response for at least 11.5 months, and 7 patients (50%) stable disease for at least 2 months. *CONCLUSIONS:* This study shows that the combination of PTK/ZK and cetuximab is well tolerated with only slightly overlapping toxicity profiles and has antitumor activity.

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Abbreviations: AE, adverse event; $AUC_{0\text{-}24}$, area under the curve 0 to 24 hours; CE(P)C, circulating endothelial (progenitor) cells; C_{max}, maximum observed plasma concentration; CTCAE, Common Terminology Criteria for Adverse Events; DLT, dose-limiting toxicity; EGFR, epidermal growth factor receptor; HER1 (R), human epidermal growth factor (receptor); IC, informed consent; MTD, maximum tolerated dose; NSCLC, non–small cell lung carcinoma; PK, pharmacokinetic; PTK787/ZK 222584 = PTK/ZK, vatalanib; small-molecule tyrosine kinase inhibitor of VEGFR-1, -2, and -3, platelet-derived growth factor receptor, cytokine stem cell factor receptor, and colony-stimulating factor 1 receptor; VEGFR, vascular endothelial growth factor receptor

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Introduction

Therapies directed against vascular endothelial growth factor (receptor) (VEGF[R]) and epidermal growth factor receptor (EGFR) have shown their clinical benefit in the treatment of cancer [1–8]. VEGF is a multifunctional cytokine that increases microvascular permeability and directly stimulates endothelial cell growth and angiogenesis [9]. The angiogenic signal is transmitted through cell surface receptors (VEGFR-1 and VEGFR-2) located on the tumor vascular endothelium. These receptors have intracellular tyrosine kinase activity. Binding of VEGF to VEGFR-2 results in the induction of several proteins, including tissue factor, urokinase, tissue-type plasminogen activator, plasminogen activation inhibitor 1, matrix metalloproteinase, and antiapoptotic factors facilitating and supporting tumor growth and tumor metastasis formation [10]. The HER1 receptor or EGFR is a prominent member of the HER growth factor receptor family. Signaling through this receptor activates a cascade that leads to proliferation, migration, survival signals, and tissue remodeling. The EGFR is overexpressed in a variety of cancers. Overexpression may range from 10% to 80% in cancer. Its expression has been associated with poor survival [11].

On the basis of the current knowledge of tumor biology, there is a rationale to combine targeted therapies that block different growth factor pathways. Although generally targeted separately, the EGFR and VEGFR pathways are interconnected. The EGFR pathway is implicated in several processes that control angiogenesis and is present on the endothelial cells of tumor vasculature [12]. Activation of EGFR leads to the induction of several angiogenic factors in tumor cells, including hypoxia-inducible factor 1 stabilization through phosphatidylinositol-3 kinase/AKT pathway activity in addition to enhanced production of VEGF by activation of the mitogen-activated protein kinase pathway. *In vitro* and *in vivo* studies show that the up-regulation of these proangiogenic factors could be inhibited by an EGFR-neutralizing antibody [13].

In addition, coexpression of EGFR and transforming growth factor α is closely associated with microvessel density in invasive cancers [14]. Preclinical studies show that inhibition of both EGFR and VEGF pathways produces additive or synergistic antitumor effects [15,16]. Finally, it has recently been published that VEGFR-1 contributes to anti-EGFR drug resistance in different human cancer cells. Interestingly, impeding VEGFR-1 activation restored sensitivity to anti-EGFR drugs [17].

Furthermore, combination treatment of enzostaurine, a protein kinase C, β_1 inhibitor, with EGFR inhibitor gefitinib, overcame resistance to EGFR inhibitors in gefitinib-resistant tumor cell lines [18]. Data from early clinical studies combining VEGF antibody bevacizumab with erlotinib in non–small cell lung carcinoma, adenocarcinoma of unknown primary, breast cancer, and renal cell cancer have been encouraging [19–23], and phase 3 trials with this combination treatment in non–small cell lung carcinoma are ongoing at this moment. Overall, it is conceivable that in malignancies being EGFR and VEGF signaling–dependant for growth and proliferation, inhibition of EGFR signaling, in combination with attenuation of VEGF-induced angiogenesis, would result in an additive/complementary or even synergistic therapeutic effect.

PTK/ZK (PTK787/ZK 222584) (vatalanib), belonging to the chemical class of aminophthalazines, is a potent and relatively selective small-molecule tyrosine kinase inhibitor of VEGFR-1, -2, and -3, platelet-derived growth factor receptor (c-Fms), cytokine stem cell factor receptor (c-kit), and colony-stimulating factor 1 receptor. PTK/ZK was evaluated in two phase 3 trials (CONFIRM 1 and 2) in advanced colorectal cancer in combination with chemotherapy [24,25]. Cetuximab (Erbitux) is a chimeric immunoglobulin G subclass 1 monoclonal antibody that blocks the binding of EGF to its receptor, inhibits cell proliferation, tumor neoangiogenesis, and metastatic potential, and promotes tumor cell apoptosis. Clinical activity has been demonstrated when given as a single agent in patients with previously treated colorectal cancer and has been approved in colorectal cancers without K-RAS mutation [4]. The combination of VEGF and EGFR inhibitors has not been extensively tested. A recently published phase 3 trial in colorectal cancer patients showed that the addition of cetuximab to capecitabine, oxaliplatin, and bevacizumab resulted in an unexpected, significantly shorter progression-free survival and inferior quality of life. Mutation status of the *KRAS* gene was a predictor of outcome in the cetuximab group [26]. Presently, there is no good explanation for this finding. It has led investigators back to the bench and the design of early clinical studies to elucidate the molecular mechanism behind the synergism or the antagonism between biologically based targeted therapies [22,27].

In this phase 1 study, we studied the safety of PTK/ZK in combination with cetuximab. Secondary objectives included determination of the pharmacokinetic (PK) profile of PTK/ZK in combination with cetuximab, definition of the optimal dosing regimen (once- or twice-daily PTK/ZK), investigation of the effect of PTK/ZK on markers of biologic activity (circulating endothelial cell measurements), and preliminary evaluation of efficacy.

Patients and Methods

Eligibility Criteria

In this single-center, phase 1 study, patients older than 18 years with advanced solid tumors refractory to or failing standard treatment were included. Patients were required to have a World Health Organization performance status of 2 or lower and adequate bone marrow, renal, and liver functions.

The most important exclusion criteria were a history of central nervous system tumors or metastases, surgery less than 10 days before the start of the study treatment or inadequate recovery from previous therapies including surgery, radiation, chemotherapy, biologic, or immunotherapy, a history of cardiac disease, congestive heart failure higher than 2 according to the New York Heart Association system, active coronary artery disease less than 6 months to study entry, cardiac arrhythmias requiring antiarrhythmic therapy, concurrent treatment with proton pump inhibitors, poorly controlled hypertension, uncontrolled infections, impairment of gastrointestinal function that may significantly alter the absorption of PTK/ZK, presence of uncontrolled diabetes, and proteinuria and patients who received experimental agents or radiation therapy within 4 weeks of the start of the study. The study was approved by the institutional ethical committee, and all patients provided written informed consent (IC). The trial was conducted in accordance with the Declaration of Helsinki.

Trial Design

In this phase 1, monocenter, open-label study conducted in a dose-escalation study design, patients were included in four cohorts of three patients with increasing dose of PTK/ZK and once- or twice-daily dosing regimens. Cetuximab was administered at a fixed dosage of 250 mg/m^2 (with a loading dose of 400 mg/m^2) weekly in all four cohorts, starting from day 8. PTK/ZK was given once (morning dose,

cohorts 1 and 2) or twice daily (morning and evening dose, cohorts 3 and 4) continuously in an escalating dose. A treatment cycle comprised 28 days. Predefined maximum dose of PTK/ZK was 1250 mg total daily based on previous single-agent studies (Table 1) [28,29]. PTK/ZK was provided by Bayer Schering Pharma (Mijdrecht, The Netherlands). In all four cohorts, patients received PTK/ZK and cetuximab until tumor progression or uncontrolled toxicity was observed. This study design allowed to simultaneously start cohorts 1 and 3 and, subsequently, cohorts 2 and 4. Toxicity was evaluated according to Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Dose-limiting toxicity (DLT) was defined as a grade 3 nonhematological adverse event (AE) related to the combination regimen or PTK/ZK alone and unrelated to the patients' underlying disease or concomitant medications, occurring during the first cycle of treatment with exception of nausea and diarrhea well controlled by intervening treatment. Neutropenia CTCAE grade 4, platelet count of 50×10^9/L or less, serum creatinine level of 2.5 × upper limit of normal or higher, proteinuria on dip stick reading CTCAE grade 2 or higher confirmed by a 24-hour urine collection with a protein level of 1.0 g or higher, hematuria CTCAE of grade 2 or higher, and suspicion of reversible posterior leukoencephalopathy syndrome were also considered to be a DLT. Toxicity solely related to the cetuximab was not considered as DLT. In case of a DLT, the cohort was expanded to six patients. The maximum tolerated dose (MTD) was defined as the dose level at which none or one of six patients experienced a DLT with at least two patients experiencing DLT at the next higher dose level. Safety review meetings were held for each cohort, before entering the next cohort. Screening assessments consisted of a complete medical history, current medication history, a complete physical examination, disease assessment, 12-lead electrocardiogram, clinical chemistry, hematology, and urinalysis. At every biweekly visit during the course of the study, a physical examination, assessment of AEs, clinical chemistry, hematology, and urinalysis was performed. Tumor assessment was performed before start of the study and every 2 months thereafter or at the discretion of the investigator. Response was assessed using the Response Evaluation Criteria in Solid Tumors guidelines [30].

PK Analysis

Blood samples for PK were collected into precooled heparinized tubes at baseline (after light breakfast) after 20 minutes and at 1, 1.5, 2, 4, 6, 8, and 24 hours after the dose of PTK/ZK, on cycle 1 day 7 (pre–cetuximab administration), day 8 (post–cetuximab administration), and on day 15 (cetuximab steady state). Within 30 minutes, plasma was prepared by centrifugation (2000*g* at 4°C for 10 minutes) and stored at −70°C until analysis. At the time of analysis, plasma monsters were diluted in blank human EDTA plasma. Plasma PTK/ZK concentrations were determined by reverse-phase high-performance liquid chromatography coupled with tandem mass spectrometry according to a validated method. Parameters to be determined were area under the concentration–time curve from the time of dosing to the last measurable concentration ($AUC_{0\text{-}24}$, calculated by linear trapezoidal summation), maximum observed plasma concentration (C_{max}), time of maximum observed plasma concentration (T_{max}), and the terminal half-life ($t_{1/2}$) using PK solutions 2.0 software (Summit Research Services, Montrose, CO). $AUC_{0\text{-}24}$ for the second gift of PTK/ZK (cohorts 3 and 4) was calculated from data from the first gift by the formula "$F \times \text{Dose} = \text{Cl} \times \text{AUC}$" with the assumption that clearance (Cl) and bioavailability (F) were unchanged.

Table 1. Study Design and Assignment of Patients.

Cohort	Dose of		No. Patients			
	PTK/ZK (Daily)	Cetuximab (Weekly)* (mg/m^2)	Screened	Enrolled†	Treated‡	DLT
1	750 mg morning dose	250	3	3	3	—
2	1250 mg morning dose	250	5	4	3	—
3	250 mg morning dose 500 mg evening dose	250	10	7	6	1
4	500 mg evening dose 750 mg morning dose	250	6	4	4	—

*First cetuximab administration after 7 days, preceded by loading dose of 400 mg/m^2.
†Patients screened who met inclusion and exclusion criteria.
‡Patients who received combination treatment for at least 7 days.

Biomarker Studies

Blood samples for the measurement of circulating endothelial (progenitor) cells (CE[P]Cs) were collected on cycle 1 day 1 predose (baseline), day 8 predose ($t = 0$) and $t = 4$ and 24 hours after cetuximab infusion, and on day 8 predose ($t = 0$) and $t = 4$ and 24 hours after cetuximab infusion. Mononuclear cells were isolated by means of an 8-ml cell preparation tube (Becton Dickinson, Breda, The Netherlands). CE(P)Cs were quantified by four color flow cytometry using CD45, CD31, CD146, and CD133 as surface markers as previously reported, defining endothelial progenitor cell as $CD45^-$, $CD31^+$, and $CD133^+$, circulating mature endothelial cell as $CD45^-$, $CD31^+$, and $CD146^+$, and progenitor cell as $CD133^+$ [31].

It was hypothesized that combination treatment with PTK/ZK and cetuximab would stabilize CE(P)C levels. Statistical comparisons between baseline and subsequent measurements were performed using the Students t test. All tests were two-sided. $P < .05$ was considered statistically significant (GraphPad Prism 4.0 [GraphPad Software, Inc, La Jolla, CA] and SPSS version 15 [SPSS, Inc, Chicago, IL]).

Results

Patient Population

A total of 24 patients were screened of which 18 patients were enrolled in the study divided over four different dose-escalating cohorts (Table 1). There were six screenings failures due to proteinuria ($n = 2$), uncontrolled hypertension, persistent anemia due to blood loss in the digestive tract, hyperbilirubinemia based on hepatic involvement, and an indication for proton pump inhibitor use due to a gastric ulcer (all $n = 1$). The median age of the patients was 61 years (range, 43-78 years). Additional patient characteristics are provided in Table 2.

Determination of the Recommended Dose

Cohort 1 (750 mg of PTK/ZK once daily) enrolled three patients (Table 1). The combination treatment at this dose level was well tolerated. Cohort 2 (1250 mg of PTK/ZK once daily) enrolled another set of three patients. No DLTs were observed, but a grade 2 proteinuria together with a grade 3 hypertension in one patient occurred. The event occurred before day 8, and this particular patient had not received combination treatment. Given that the proteinuria was already preexistent at the start of the study (note to file) and the hypertension was immediately well managed with antihypertensive treatment, these events were both considered not DLTs. This patient was replaced. Taken together, no DLTs were observed at this dose level.

Table 2. Patient Characteristics.

	No.
No. patients enrolled	18
Sex	
Male	10
Female	8
Age (years)	
Median	61
Range	43-78
WHO status	
0	12
1	5
2	1
Primary tumor	
Colorectal cancer	10
Cholangiocarcinoma	1
Pancreatic cancer	1
Bronchus carcinoma	1
Chordoma	2
Breast cancer	1
Ovarian cancer	1
Cervix uteri carcinoma	1
No. metastatic sites	
1	3
2	3
3	4
4	4
5	2
≥6	2
Prior therapy	
Surgery	15
Systematic therapy	18
Radiotherapy	6
No. previous chemotherapy regimens	
1	1
2	6
3	5
≥4	2
Time from first diagnosis (years)	
Median	1.8
Range	0.1-13.1

WHO indicates World Health Organization.

In cohort 3 (750 mg of PTK/ZK twice daily), initially three patients were enrolled. No DLTs were observed. However, several likely PTK/ZK–related grade 2 and three grade 3 AEs occurred (liver biochemistry disturbances, hypertension, and deep venous thromboembolism) just beyond the predefined safety period of 28 days. Therefore, it was decided by the investigators to expand this cohort to six patients. In these additional three patients, one DLT occurred (aspartate aminotransferase [AST] increase grade 3). One patient with a grade 4 anaphylactic reaction directly after the first cetuximab infusion was replaced. In addition, one patient presented with grade 3 acne likely because of noncompliance of the patient to the recommended dermatological supportive care. The skin toxicity quickly reversed to grade 1 with structured and intensive dermatological support. On the basis of these findings, the investigators decided that it was safe to enroll patients at the final dose level.

Cohort 4 (1250 mg of PTK/ZK twice daily) included four patients in total. One patient with pancreatic cancer withdrew IC after 14 days of treatment because of symptoms of a preexistent mood disorder. This patient was replaced. No DLTs occurred at this dose level. A grade 3 increase in γ-glutamyl transpeptidase and a grade 3 hyponatremia and tachycardia (one patient) were observed. However, because these events were unrelated to the study treatment or already existent at the start of the study, they were not considered DLTs. The study was terminated at this dose level because the recommended dose for PTK/ZK from single-agent phase 1 studies was attained.

Safety and Tolerability

Of all 18 patients assigned to study treatment, 16 patients received combination therapy and therefore were assessable for safety analysis. Two patients went off the study before or during the first cetuximab administration. Overall, the combination of PTK/ZK and cetuximab was well tolerated.

Drug-related AEs (all grades) reported by more than 30% of the patients were acne (87%), dry skin (81%), fatigue (69%), nausea (63%), dizziness (50%), vomiting (50%), headache (38%), diarrhea (31%), fissures of the skin (31%), and hypertrichosis (31%). Frequencies of treatment-related AEs CTCAE grade 2 and 3 are presented in Table 3. Grade 3 toxicity was most frequently observed in cohorts 3 and 4. During the whole study, only one DLT consisting of a grade 3 increase in AST and only one CTCAE grade 4 toxicity comprising an anaphylactic reaction after cetuximab administration were reported. This anaphylactic reaction was well controlled with immediate intervention (epinephrine and antihistamines) and was undoubtedly cetuximab-related. Serious adverse events possibly related to study treatment consisted of pneumonia and a pneumothorax and resolved well after admission for intravenous antibiotic treatment and pleural drainage, respectively.

Treatment-related skin toxicity (all CTCAE grades) was observed in 15 of the 16 evaluable patients. These AEs were mainly classified as CTCAE grade 2/3 and were well manageable with structured and intensive dermatological support.

Fatigue was the second most frequently reported symptom (69%, CTCAE grade 1 $n = 8$; CTCAE grade 2 $n = 3$), interfering with daily activities while on treatment, but not being a reason to discontinue

Table 3. Combination Treatment Emergent–Related AEs CTCAE Grade 2 and 3.

Toxicity	Toxicity Grade (No. Patients)*									
	Grade 2					Grade 3				
	Cohort				Total (%)	Cohort				Total (%)
	1	2	3	4		1	2	3	4	
Abscess	1	0	0	0	1 (6)	0	0	0	0	0
Acne	0	0	1	2	3 (19)	0	1	0	0	1 (6)
Cardiac ischemia	0	0	1	0	1 (6)	0	0	0	0	0
Dizziness	0	1	1	0	2 (12)	0	0	0	0	0
Dry skin	1	0	1	0	2 (12)	0	0	0	0	0
VTE	0	0	0	0	0	0	0	1	0	1 (6)
Fatigue	0	0	2	1	3 (19)	0	0	0	0	0
Headache	0	0	1	0	1 (6)	0	0	0	0	0
Hypertension	0	1	0	1	2 (12)	0	0	2	0	2 (12)
Nausea	0	0	1	1	2 (12)	0	0	0	0	0
Neutropenia†	1	0	0	0	1 (6)	0	0	1	0	1 (6)
Proteinuria†	0	1	1	0	2 (12)	0	0	0	0	0
Heartburn	0	0	1	0	1 (6)	0	0	0	0	0
Rash perianal	0	0	1	0	1 (6)	0	0	0	0	0
Transaminitis (ALT)†	1	1	1	0	3 (19)	1	0	2	0	3 (19)
Transaminitis (AST)†	2	1	2	0	4 (25)	0	0	1‡	2	3 (19)
Vomiting	0	0	1	1	2 (12)	0	0	0	0	0
Total AE	6	5	15	6		1	1	6	2	

ALT indicates alanine transaminase; *VTE*, venous thromboembolism.
*Sixteen patients were assessable for safety analysis.
†For laboratory/metabolic disturbances, all emerging AEs are displayed.
‡DLT for that cohort.

study medication. Hypertension occurred in 4 (25%) of the 16 patients (CTCAE grade 2 $n = 2$; CTCAE grade 3 $n = 2$) and was well controlled by a standardized hypertension management protocol, commencing with a calcium channel blocker followed, when needed, by a β-blocker or by an angiotensin-converting enzyme inhibitor. Dizziness occurred in 8 (50%) of the 16 patients (CTCAE grade 1 $n = 6$; CTCAE grade 2 $n = 2$). The total reported related AEs (all CTCAE grades) for the four successive cohorts were 57, 63, 121, and, 80 respectively.

The median number of days on treatment for all 16 patients in the four cohorts was 49 (range, 34-214), 134 (range, 7-344), 48 (range, 6-331), and 46 (range, 14-57), respectively. Eleven patients discontinued study permanently owing to progressive disease, four patients owing to toxicity (liver disturbances $n = 2$, cetuximab-related anaphylaxis $n = 1$, pneumothorax $n = 1$), and one patient withdrew IC because of multiple toxicity (Table 4). Dose intensities for both the study treatments differed slightly between the once- and twice-daily cohorts in favor of the once-daily regimens. Reductions were equally distributed among all cohorts, and there were no clear differences in dose delays and interruptions between the once- and twice-daily dosing regimens and between the two dosage groups (Table 4).

PK and Biomarker Studies

Seventeen patients were evaluable for PK analysis. PK profiles composed for PTK/ZK are shown in Figure 1. PTK/ZK was rapidly absorbed after oral administration, with the C_{max} reached in approximately 1 to 2 hours and mean half-life of PTK/ZK of 5 hours. No significant changes in PTK/ZK exposure on coadministration with cetuximab (PK profile days 7 and 8) were observed. Incidental changes observed were of low magnitude and within the usual range of interpatient variability. At equivalent total daily doses, no statistically significant differences in systemic exposure ($AUC_{0\text{-}24}$) for once- and twice-daily dosing cohorts were observed. There was a statistically significant difference in C_{max} between cohorts 2 and 4 on day 7 ($P < .0017$) and day 8 ($P < .04$; Student's t test). No statistically significant differences were found in PK profiles between day 7 (PTK/ZK only), day 8 (combination PTK/ZK and cetuximab), and day 15 (steady-state cetuximab). Measurements of (circulating) endothelial (progenitor) cells by flow cytometry analysis showed no significant changes in endothelial progenitor cell, circulating mature endothelial cells, and progenitor cell levels. There was no correlation found between response to therapy and levels of (C)(E)(P)Cs (Figure 2).

Table 4. Treatment Administration Summary*.

	Cohort			
	1	2	3	4
Dose PTK/ZK (mg)				
Morning	750	1250	250	500
Evening			500	750
Dose intensity (mean)†				
PTK/ZK	0.94	0.88	0.77	0.81
Cetuximab	0.94	0.93	0.85	0.72
Days on treatment				
Median (range)	49 (34-214)	134 (7-344)	48 (6-331)	46 (14-57)
Dose PTK/ZK				
Reduction‡	0	1	1	0
Delay§	0	2	1	0
Temporarily interrupted¶	0	2	1	0
Permanently interrupted	1	1	5	1
Reason delay/interruptions				
Toxicity	1	4	5	1
Logistical	0	1	1	0
Hypersensitivity reaction	0	0	1#	0
Dose cetuximab				
Reduction‡	0	1	0	0
Delay§	0	2	1	0
Temporarily interrupted¶	0	3	1	0
Permanently interrupted	1	1	4	1
Reason delay/interruptions				
Toxicity	1	4	4	1
Logistical	0	2	1	0
Hypersensitivity reaction	0	0	1#	0

*All enrolled patients included in the analysis ($n = 18$).
†Defined as proportion of planned dose to receive and dose received during 2 months of treatment.
‡Reduction of more than 10%.
§Delay of more than 3 days.
¶Temporarily interruptions defined as a delay of more than 3 days.
#Anaphylaxis to cetuximab.

Disease Response

Of the 18 patients assigned to study treatment, 14 patients were evaluable for efficacy analysis. Reasons for invalidity were early discontinuation before combination therapy was administered owing to toxicity ($n = 2$, anaphylactic reaction and proteinuria) and premature discontinuation during combination therapy which impeded evaluation ($n = 2$, withdrawal of IC and grade 3 transaminitis). All patients entered into the study had progressive disease at time of enrollment.

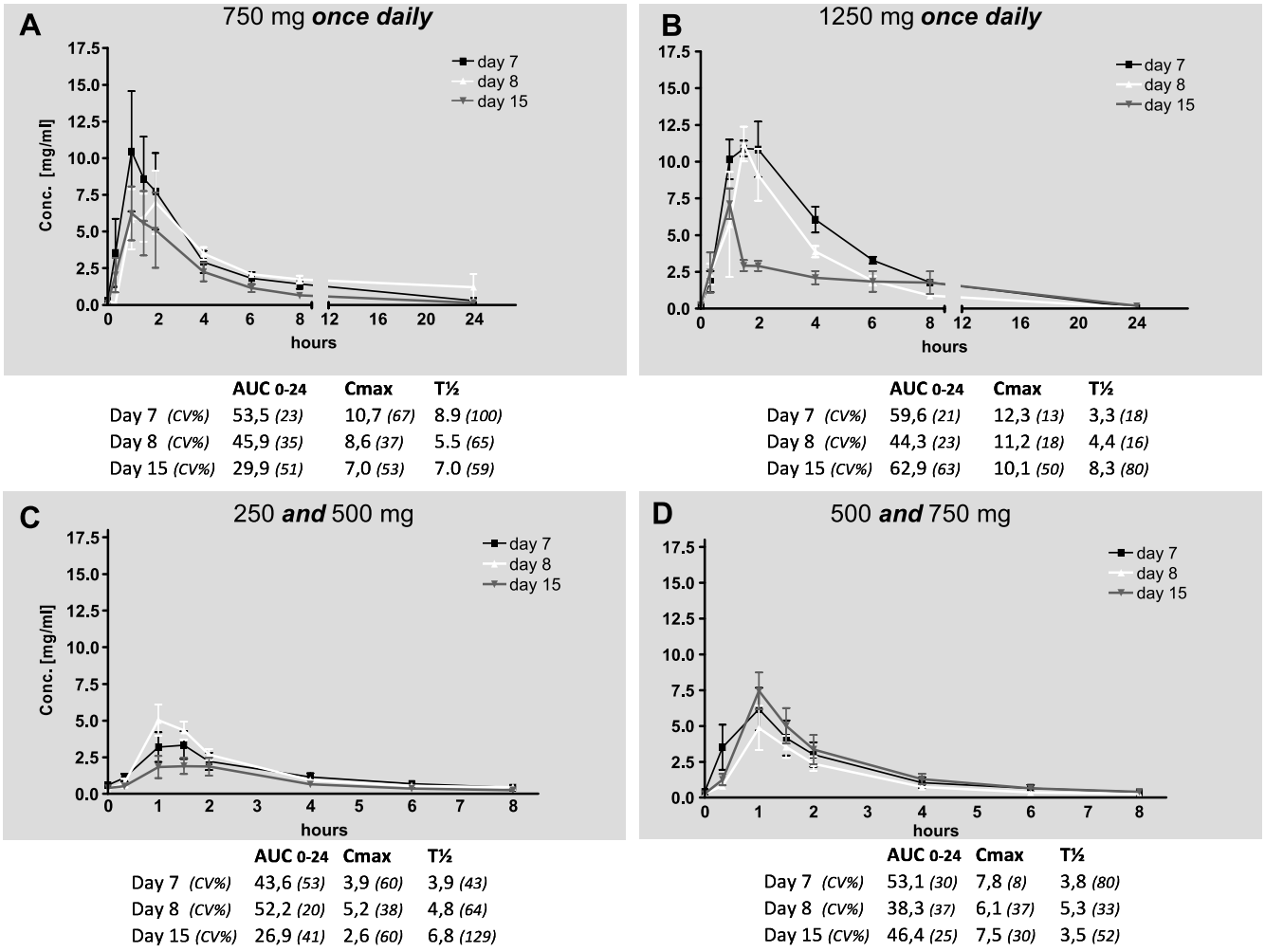
Clinical benefit was observed in eight of the 14 assessable patients (57%). Stable disease for 2 months or longer was seen in 7 (50%) of the 14 assessable patients (colorectal cancer $n = 2$, chordoma $n = 2$, cholangiocarcinoma $n = 1$, breast cancer $n = 1$, and cervix carcinoma $n = 1$) with a median time on study of 4.5 months (range, 2-12 months). Confirmed and sustained partial response (–40%) with a duration of 11.5 months was observed in one patient with colorectal cancer.

Discussion

In phase 1/2 PTK/ZK or cetuximab single-agent trials, treatment was well tolerated. The MTD for PTK/ZK monotherapy was defined as 1250 mg once daily. On the basis of these results and the only marginally overlapping toxicity profiles of the single agents, the combination of cetuximab and PTK/ZK in escalating cohorts from subtherapeutic to therapeutic dose was hypothesized to be tolerable and safe. The results from this current study indeed confirm that combining PTK/ZK to cetuximab in therapeutical dosages is a safe and well-tolerated combination treatment with possible biologic activity.

The toxicity profile of the combination treatment was mainly consistent with the toxicity as reported in the previously mentioned single-agent trials and consisted mainly of the known toxicities caused by the agents individually with a trend to complementary frequencies for certain AEs (acne, fatigue, nausea, and vomiting) [28,29]. The combination of PTK/ZK and cetuximab did not seem to increase the severity of these well-known toxicities caused by each agent individually. Hypertension occurred at a frequency one would expect for a VEGF inhibitor of this class, and the occurrence of skin toxicity was slightly increased to observed frequencies in previous studies with cetuximab [32–34].

Both AEs could be well managed with strict and structured intervention protocols, and they did not jeopardize the therapeutic potential of the treatment.



	AUC 0-24	Cmax	T½
Day 7 *(CV%)*	53,5 *(23)*	10,7 *(67)*	8.9 *(100)*
Day 8 *(CV%)*	45,9 *(35)*	8,6 *(37)*	5.5 *(65)*
Day 15 *(CV%)*	29,9 *(51)*	7,0 *(53)*	7.0 *(59)*

	AUC 0-24	Cmax	T½
Day 7 *(CV%)*	59,6 *(21)*	12,3 *(13)*	3,3 *(18)*
Day 8 *(CV%)*	44,3 *(23)*	11,2 *(18)*	4,4 *(16)*
Day 15 *(CV%)*	62,9 *(63)*	10,1 *(50)*	8,3 *(80)*

	AUC 0-24	Cmax	T½
Day 7 *(CV%)*	43,6 *(53)*	3,9 *(60)*	3,9 *(43)*
Day 8 *(CV%)*	52,2 *(20)*	5,2 *(38)*	4,8 *(64)*
Day 15 *(CV%)*	26,9 *(41)*	2,6 *(60)*	6,8 *(129)*

	AUC 0-24	Cmax	T½
Day 7 *(CV%)*	53,1 *(30)*	7,8 *(8)*	3,8 *(80)*
Day 8 *(CV%)*	38,3 *(37)*	6,1 *(37)*	5,3 *(33)*
Day 15 *(CV%)*	46,4 *(25)*	7,5 *(30)*	3,5 *(52)*

Figure 1. PK analysis for PTK/ZK per cohort: once- (A and B) and twice-daily dosing cohorts (C and D). *AUC* indicates area under the curve (mg h/L); C_{max}, maximum observed plasma concentration (mg/L); $t_{1/2}$, half-life (hours); Day 7 (after 7 days of PTK/ZK exposure), Day 8 (PTK/ZK and cetuximab), Day 15 (steady state of combination).

Only one DLT occurred in this study, in cohort 3, consisting of an AST increase. Also, the fact that four patients were on treatment for more than 7 months further endorses the fair tolerability of the combination. On the basis of the comparison between the once- and twice-daily cohorts for the parameters total reported AEs, the median days on treatment, and the dose intensities, once-daily dosing regimens seemed to be slightly better tolerated than twice-daily regimens. Consistent with previously published studies, PK analysis

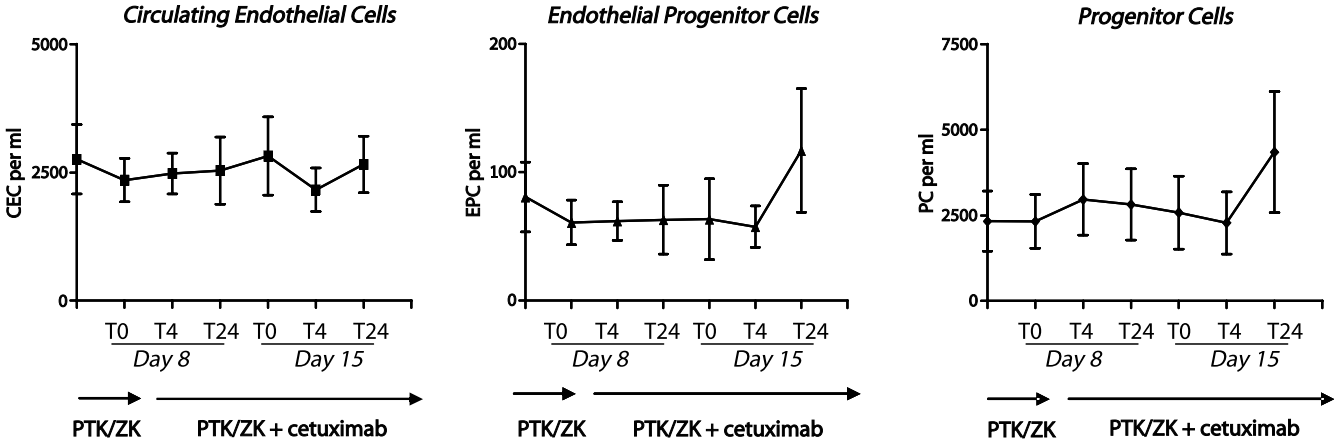


Figure 2. Measurement of (circulating) (endothelial) (progenitor) cells (C)(E)(P)Cs at various predefined time points by flow cytometry analysis. T_0 indicates baseline before PTK/ZK and cetuximab administration; $T_{4/24}$, 4/24 hours after PTK/ZK administration.

revealed no significant changes in PTK/ZK bioavailability at equivalent total daily doses [28]. As a result, once-daily dosing might be preferable than twice-daily dosing.

The study was terminated after dose level 4 because the recommended doses for PTK/ZK and cetuximab from single-agent phase 1 studies were attained. No MTD was defined.

As a result, together with the comparisons between once- and twice-daily dosing levels, the optimal treatment regimen for the combination was defined as the dose level with the predefined maximum combination dosages: PTK/ZK 1250 mg and cetuximab 250 mg/m^2. Consequently, it seems to be appropriate to conclude that once-daily dosing might be preferable to twice-daily dosing.

Analysis of endothelial (progenitor cells) (E(P)C) levels showed stabilized levels during the course, possibly suggesting that administration of PTK/ZK might blunt the expected higher baseline levels in patients with progressive disease [35,36].

The absence of additional time points with PTK/ZK only and the heterogeneous composition of our small patient population prohibit a definitive conclusion on this part and the findings should be considered as exploratory.

There was no correlation between disease status or response to therapy and levels of (C)(E)(P)Cs. This was largely due to great variability, possibly related to the heterogeneity and the extensive pretreatment of the study population.

In conclusion, this study reveals that dual targeting of the VEGFR and EGFR pathway by means of combining PTK/ZK and cetuximab was well tolerated at relevant single-agent doses of both agents, and antitumor activity was found in severely pretreated patients. These results represent a first proof of concept of combining safely an EGF-inhibiting antibody and a VEGFR tyrosine kinase inhibitor and support further preclinical and early clinical research concerning the combination of EGFR and VEGF-inhibiting treatment in malignancies although supported by well-designed hypertension management and dermatological care strategies.

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