Soluble markers for the assessment of biological activity with PTK787/ZK 222584 (PTK/ZK), a vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor in patients with advanced colorectal cancer from two phase I trials


1Tumor Biology Center, Freiburg; 2ProQuinase GmbH, Freiburg, Germany; 3Translational and Clinical Development, Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA; 4Preclinical Research, Novartis Pharma, Basel, Switzerland; 5Leceister Royal Infirmary Hospital, Leicester, UK; 6Schering AG, Berlin, Germany

Received 27 August 2004; revised 16 November 2004; accepted 17 November 2004

Background: Plasma and serum biomarkers of angiogenesis and activated endothelial cells were evaluated to assess biological activity of PTK787/ZK 222584 (PTK/ZK), a novel oral angiogenesis inhibitor targeting all known vascular endothelial growth factor (VEGF) receptor tyrosine kinases.

Patients and methods: Patients with colorectal cancer (CRC) (n = 63) were enrolled into two phase I/II dose escalation trials of PTK/ZK in 28-day cycles until discontinuation. Patients with stable disease for ≥2 months were categorized as ‘non-progressors’. Plasma markers of angiogenesis, VEGF-A and basic fibroblast growth factor (bFGF), and the serum markers of activated endothelial cells, sTIE-2 and sE-Selectin, were assessed at baseline, and pre-dose on days 1, 8, 15, 22 and 28 of every cycle, with additional assessments 10 h post-dose on days 1 and 15. The percentage change from baseline was subsequently correlated with AUC and C_{max} of PTK/ZK on day 1, cycle 1 and clinical outcome.

Results: A dose-dependent increase in plasma VEGF-A and bFGF was observed in the first cycle of PTK/ZK treatment. The correlation of change in plasma VEGF-A with AUC and C_{max} was characterized by an E_{max} model, suggesting that a change of ≥150% from baseline VEGF-A correlated with non-progressive disease. Change from baseline plasma VEGF-A within the first cycle of treatment was significantly correlated with clinical outcome by logistic regression analysis (P = 0.027).

Conclusions: In patients with CRC treated with PTK/ZK, changes in plasma VEGF-A and bFGF demonstrate biological activity of PTK/ZK, may help to establish optimal dose and correlate with outcome.

Key words: angiogenesis, biomarker, VEGF, VEGF receptor inhibitor

Introduction

Antiangiogenic therapy is a promising new strategy for inhibiting tumor growth and metastasis [1]. Recently, a number of antiangiogenic agents have entered clinical trials [2]. Unlike cytotoxic drugs, antiangiogenic agents target host endothelial cells and are expected to be predominantly cytostatic rather than directly cytotoxic, although some tumor regression may occur. For conventional cytotoxic anticancer agents, the optimal dose has usually been defined as the maximum tolerated dose (MTD). In contrast, antiangiogenic agents may achieve maximum therapeutic effect at doses well below the MTD. Therefore, it is important to assess quantifiable effects on the molecular target or biological parameters downstream from the molecular target, as well as safety end points to establish the dose–effect relationship and determine both the optimal biological dose (OBD) and the MTD. Biomarkers are essential to establish biological activity and determine the OBD [3].

Tumor regression is usually measured by standard non-invasive imaging techniques such as computed tomography.
(CT) and magnetic resonance imaging (MRI). Antiangiogenic agents may not induce immediate tumor regression; therefore, traditional imaging techniques provide a limited assessment of biological activity. Biomarkers, however, have been used successfully to assess biological activity in response to antiangiogenic agents. Dynamic contrast-enhanced MRI (DCE-MRI), while its availability is limited, appears to be a promising biomarker for assessing early changes in tumor-associated vasculature in response to treatment with antiangiogenic agents [4].

Soluble plasma and serum markers of angiogenesis and of activated endothelial cells can also be used to assess antiangiogenic activity. We have investigated several soluble biomarkers, including a member of the vascular endothelial growth factor family (VEGF-A), basic fibroblast growth factor (bFGF) and several endothelial cell-associated molecules. Members of the VEGF family (VEGF-A, -B, -C and -D) play a critical role in angiogenesis [5]. In addition to increasing vascular permeability, the family of VEGF ligands stimulate endothelial cell proliferation, migration and tube formation [6]. The effects of each VEGF ligand are mediated through receptors, VEGFR-1, -2 and -3, located on endothelial cells. bFGF is also an endothelial cell-specific angiogenic factor that plays a key role in tumor angiogenesis [7]. The family of VEGF proteins and bFGF are produced by tumor cells in response to tumor hypoxia. Therefore, the concentration of these angiogenic factors in the plasma may reflect the hypoxic status of a tumor and may increase in response to effective blockade of VEGF receptors on endothelial cells.

Endothelial cell-associated molecules that are shed into the plasma can provide an indirect measure of the amount of newly forming vessels in a patient’s tumor. E-Selectin is an endothelial cell adhesion molecule, and the soluble form of E-Selectin (sE-SEL) may increase tumor angiogenesis and the adhesion of tumor cells to endothelial cells at distant sites [8]. The transmembrane tyrosine kinase TIE-2, the receptor for the angiopoietin-1 and -2, is also involved in angiogenic processes and is ubiquitously expressed on endothelial cells throughout the vasculature. The soluble form of TIE-2 (sTIE-2) can be detected in serum from healthy individuals [9, 10].

We have used these biomarkers to assess the biological activity of PTK787/ZK 222584 (PTK/ZK), a novel oral angiogenesis inhibitor (co-developed by Novartis and Schering AG, Berlin) that selectively inhibits the phosphorylation of all known VEGF receptor tyrosine kinases, as well as the platelet-derived growth factor receptor and c-kit, to a lesser degree. Here we report the effects of PTK/ZK on plasma/serum concentrations of soluble biomarkers, including VEGF-A and bFGF, sE-SEL and sTIE-2, in patients with colorectal cancer (CRC).

Patients and methods

Patient eligibility criteria

Results are from a pooled analysis of patients with advanced CRC enrolled into two phase I/II clinical trials. Study PTK787/ZK 222584 CPTK787 0101 (Study 0101) enrolled patients with a variety of advanced cancers, whereas study PTK787/ZK 222584 CPTK787 0103 (Study 0103) enrolled patients with advanced colorectal and breast cancers with liver metastases.

Patients with histologically confirmed advanced solid malignancies with no standard curative therapy were eligible. All patients were required to have at least one site of measurable or evaluable disease as determined by the Southwest Oncology Group (SWOG) criteria. Inclusion was irrespective of stage of disease or extent of prior therapy. Patient entry criteria included: age ≥18 years; WHO performance status of 0–2; adequate hematomatological (absolute neutrophil count ≥1.5 × 10⁹/l, hemoglobin ≥9 g/dl, platelets ≥100 × 10⁹/l), renal [serum creatinine ≤1.5 × upper limit of normal (ULN)], serum bilirubin ≤1.5 × ULN, 24-h creatinine clearance ≥50 ml/min] and hepatic [aspartate aminotransferase and alanine aminotransferase ≤2.5 × ULN] function; no known brain metastases; no recent prior chemotherapy or biological therapies, radiotherapy or surgery; and a life expectancy of at least 12 weeks.

All patients were informed about the investigational nature of the study according to institutional and regional guidelines, and provided written consent before beginning therapy. Permission of local ethics regulatory bodies was obtained at each center.

Response criteria

Patients were evaluated for tumor response at the end of every 28-day cycle using the SWOG Solid Tumor Response Criteria. All measurable and non-evaluable lesions were accounted for in the tumor assessment. Measurable lesions were quantified using the product of perpendicular diameters. Methods for assessment varied between patients (but were consistent for each patient throughout the course of study) and included physical examination, laboratory values, ultrasound and MRI. Minor response (MR) was defined as a ≥25% but ≤50% decrease from baseline in measurable lesions. Progressive disease (PD) was defined as a ≥50% increase or an increase of 10 cm² (whichever was smaller) in measurable lesions, clear worsening from previous assessment of any evaluable disease, reappearance of any lesion which had disappeared or appearance of any new lesion/site. Stable disease (SD) was defined as not meeting the criteria for either MR or PD.

Best response criteria were used to categorize all evaluable patients as either non-progressors or progressors for the surrogate marker analysis to identify the differences in biological effects in response to PTK/ZK efficacy. The best response was determined retrospectively from two consecutive evaluations 28 days apart. Patients with either MR or SD on two consecutive evaluations were prospectively defined as non-progressors; no patient achieved a complete or partial response.

Study design, drug administration and biomarkers

PTK/ZK was administered orally once daily in 28-day cycles until discontinuation secondary to adverse events or tumor progression. Dose levels are shown in Table 1. Three patients were enrolled per dose cohort, and an additional three patients were enrolled at the same dose level in the event that a dose-limiting toxicity was observed. An additional six to 25 patients were enrolled at the dose level defined as optimal (with respect to toxicity, pharmacokinetic and biomarkers) for further assessment of biological activity and safety (dose expansion cohort).

Plasma and serum samples were obtained for measurement of potential biomarkers of angiogenesis (VEGF-A, bFGF, sTIE-2 and sE-SEL) in every treatment cycle. All samples were collected at baseline and at designated time points immediately before dosing (days 1, 8, 15, 22 and 28),
AUC Corporation, Mountain View, CA, USA), AUC0–tration. Using non-compartmental methods (WinNonlin Pro 3.2; Pharsight on day 1 and at EC1 by visual inspection of each patient’s plasma concen-

points on the terminal log-linear portion of the plasma concentration–time was calculated by the linear regression of the WinNonlin selected data and then extrapolated to infinity. AUC 0–24 was calculated on EC1 using 1 using the linear trapezoidal rule up to the last measurable data point, was calculated by dividing 0.693 by \( t \) the linear trapezoidal rule up to 24 h post-dose. The

Starting dose 50 mg \( (n=3, \text{CRC}=3) \)
150 mg \( (n=3, \text{CRC}=2) \)
300 mg \( (n=3, \text{CRC}=0) \)
500 mg \( (n=6, \text{CRC}=3) \)
750 mg \( (n=3, \text{CRC}=0) \)
1000 mg \( (n=6, \text{CRC}=4) \)
1200 mg \( (n=13, \text{CRC}=10) \)

1500 mg \( (n=11, \text{CRC}=2) \)

Highest dose 2000 mg \( (n=3, \text{CRC}=3) \)

CRC = colorectal cancer.

with the exception of two samples taken 10h post-dose on days 1 and 15 (Figure 1). Full pharmacokinetic analysis was only conducted on days 1, 15 and 28 of cycle 1.

PTK/ZK bioanalytical assay

PTK/ZK assay was performed using high-performance liquid chromatography. At a concentration of 20, 800 and 8000 ng/ml, the intraday variabil-

ity [coefficient of variation (CV) %] was 0.7, 1.4 and 1.3, respectively, and the interday variability (CV%) was 1.3, 2 and 1.6, respectively. The CV for the assay sensitivity at 2.5 ng/ml is 4.

PTK/ZK pharmacokinetic assessment

Full pharmacokinetic parameters were obtained on days 1, 15 and 28 in the cycle 1 at the following time points: pre-dose (0), and 0.25, 0.5, 1, 1.5, 2, 4, 6, 10 and 24 h post-dose. The pharmacokinetic parameters, area under the plasma concentration curve (AUC), maximum plasma concentration (\( C_{\text{max}} \)), minimum plasma concentration (\( C_{\text{min}} \)) and elimination half-life (\( t_2 \)) were determined for each individual plasma concentration–time data on day 1 and end of cycle 1 (EC1). The \( C_{\text{max}} \) and \( C_{\text{min}} \) were determined on day 1 and at EC1 by visual inspection of each patient’s plasma concentration. Using non-compartmental methods (WinNonlin Pro 3.2; Pharsight Corporation, Mountain View, CA, USA), AUC\(_{0\rightarrow\infty}\) was calculated on day 1 using the linear trapezoidal rule up to the last measurable data point, and then extrapolated to infinity. AUC\(_{0\rightarrow24}\) was calculated on EC1 using the linear trapezoidal rule up to 24 h post-dose. The \( t_2 \) on day 1 and EC1 was calculated by dividing 0.693 by \( k_e \) (elimination rate constant), which was calculated by the linear regression of the WinNonlin selected data points on the terminal log-linear portion of the plasma concentration–time curve.

Biomarker bioanalytical methodology

Plasma samples were assayed for VEGF-A, bFGF and sE-SEL using the relevant quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems Europe, Oxford, UK) according to the manufacturer’s instructions. All samples and standards were assayed in duplicate. sTIE-2 serum levels were determined by ELISA as described by Reusch et al. [8].

Biomarker assessment

The units for the biomarker concentration were as follows: VEGF-A [limit of quantitation (LOQ) 31.25] and bFGF (LOQ 1.00) were expressed in pg/ml; sTIE-2 and sE-SEL (LOQ 0.20) were expressed in ng/ml. All values below the limit of quantitation were set to the LOQ for the biomarker analysis.

Changes in plasma and serum markers in response to PTK/ZK treat-

ment were evaluated in the first cycle of treatment as indicators of biologi-

cal activity. Because of high baseline variability, data were expressed as percentage of baseline and subsequently averaged across the first cycle of treatment. Patients who met the following inclusion criteria for biomarker analysis were included in the analysis: evaluable disease status of ‘non-progressive’ or ‘progressive’ disease; enrolled on either the dose escalation or expansion groups of the trial; treated with PTK/ZK on the day of biomarker sampling; and must have at least five of the seven data points in the first 28 days of PTK/ZK treatment.

Pharmacokinetic biomarker data: tumor response analysis and statistical analysis

To characterize the relationship between mean change from baseline plasma/serum marker versus PTK/ZK exposure (AUC) and maximum PTK/ZK concentration (\( C_{\text{max}} \)) on day 1, pharmacodynamic modeling was performed by fitting the data to an \( E_{\text{max}} \) model. The pharmacokinetic data from day 1, instead of EC1, was used in the modeling to allow for a wider range of exposure and concentration to be characterized in the exposure/ concentration versus effect curve.

\[
\text{Effect} = E_0 + (E_{\text{max}} - E_0) \times \frac{AUC}{AUC + EAUC_{50}}
\]

where Effect = average % of baseline plasma/serum marker; \( E_0 = \) approximated baseline (~100%), expressed as % of baseline plasma/ serum marker; \( EAUC_{50} = AUC \) in which 50% of \( E_{\text{max}} \) is achieved; \( E_{\text{max}} = \) maximum effect, expressed as % of baseline plasma/ serum marker.

A uniform weighting scheme was used. The final model and parameters were selected based on visual inspection, statistical estimation of the goodness of fit and an understanding of the biology with antiangiogenic agents. Furthermore, to assess the correlation between clinical outcome

Table 1. Dose escalation scheme from two phase I/II clinical trials \( (n=63, \text{CRC}=38) \)

<table>
<thead>
<tr>
<th>Trial 1 ( (n=38, \text{CRC}=17) )</th>
<th>Trial 2 ( (n=25, \text{CRC}=21) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting dose</td>
<td>50 mg ( (n=3, \text{CRC}=3) )</td>
</tr>
<tr>
<td></td>
<td>300 mg ( (n=3, \text{CRC}=0) )</td>
</tr>
<tr>
<td></td>
<td>750 mg ( (n=3, \text{CRC}=0) )</td>
</tr>
<tr>
<td></td>
<td>1200 mg ( (n=13, \text{CRC}=10) )</td>
</tr>
</tbody>
</table>

CRC = colorectal cancer.

Figure 1. Soluble markers sampling schedule.
and change in plasma or serum markers, the data were fit to a logistic regression model.

\[
\text{Pr} \text{ (non-progressor)} = \frac{e^{(a + b^*x)}}{1 + e^{(a + b^*x)}}
\]

where \( \text{Pr} \text{ (non-progressor)} = \) probability of achieving “non-progressive” disease, \( a = \) constant, \( b = \) coefficient of predictor variables, and \( x = \) average % of baseline plasma or serum marker.

The relationship between change from baseline for biomarkers and PTK/ZK dose and clinical outcome were analyzed using regression analysis (S-PLUS, version 2.0; Mathsoft Engineering & Education, Cambridge, MA, USA). The degree of correlation between pharmacodynamic parameters and change from baseline biomarkers was assessed using Spearman Rank correlations. Significance was assigned at \( P < 0.05; P < 0.10 \) was described as a trend.

**Results**

**Patient characteristics**

Sixty-three \((n = 38 \text{ CRC})\) patients were enrolled in the two phase I/II clinical trials (Table 1). Of the 38 CRC patients, 30 met the inclusion criteria and were included in the soluble marker analysis. Patient demographics are listed in Table 2. Reasons for excluding eight patients from the analysis were: disease status not evaluable \((n = 6)\), extreme sample hemolysis \((n = 1)\) and re-entrant to study \((n = 1)\). Six patients were not evaluable for disease status for the following reasons: discontinuation due to reasons other than PD or adverse events \((n = 4)\) and pending tumor response \((n = 2)\). Of the 30 CRC patients included in the biomarker analysis, 16 patients had non-progressive disease (i.e. MR or SD) and 14 patients had progressive disease. Of the 16 non-progressors, the majority \((n = 12; 75\%)\) received \(\geq 1000\text{ mg/day of PTK/ZK}\). Of the 14 progressors, the majority \((n = 9; 65\%)\) received \(\leq 1000\text{ mg/day of PTK/ZK}\) treatment. All non-progressors received \(\geq 750\text{ mg/day of PTK/ZK}\) treatment.

**Pharmacokinetic assessment**

The mean pharmacokinetic parameters, AUC and \(C_{\text{max}}\), are listed by dose groups and by day 1 and EC1 in Table 3.

**Table 3.** Mean \((\pm \text{ standard deviation})\) pharmacokinetic parameters (AUC, \(C_{\text{max}}\)) by dose groups on day 1 and end of treatment cycle 1 (EC1)

<table>
<thead>
<tr>
<th>Dose ((\text{mg/day}) [n])</th>
<th>(\text{AUC}_{0-\infty}) ((\text{day 1}))</th>
<th>(\text{AUC}_{0-24}) ((\text{EC1}))</th>
<th>(C_{\text{max}}) ((\text{day 1}))</th>
<th>(C_{\text{max}}) ((\text{EC1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ((3/3))</td>
<td>(6.20 \pm 5.65)</td>
<td>(7.78 \pm 6.28)</td>
<td>(1.60 \pm 1.49)</td>
<td>(2.38 \pm 1.54)</td>
</tr>
<tr>
<td>150 ((2/2))</td>
<td>(18.49 \pm 7.67)</td>
<td>(15.42 \pm 2.43)</td>
<td>(5.87 \pm 1.23)</td>
<td>(4.69 \pm 2.61)</td>
</tr>
<tr>
<td>300 ((3/3))</td>
<td>(15.19 \pm 6.94)</td>
<td>(14.97 \pm 7.00)</td>
<td>(5.20 \pm 2.77)</td>
<td>(5.22 \pm 2.92)</td>
</tr>
<tr>
<td>500 ((4/4))</td>
<td>(78.90 \pm 26.20)</td>
<td>(46.89 \pm 3.43)</td>
<td>(13.02 \pm 3.38)</td>
<td>(13.05 \pm 2.86)</td>
</tr>
<tr>
<td>750 ((3/3))</td>
<td>(94.28 \pm 83.66)</td>
<td>(56.97 \pm 21.24)</td>
<td>(16.91 \pm 9.36)</td>
<td>(15.40 \pm 5.15)</td>
</tr>
<tr>
<td>1000 ((7/7))</td>
<td>(236.46 \pm 73.85)</td>
<td>(80.86 \pm 34.62)</td>
<td>(25.19 \pm 6.10)</td>
<td>(18.61 \pm 7.03)</td>
</tr>
<tr>
<td>1200 ((4/4))</td>
<td>(214.50 \pm 187.21)</td>
<td>(120.55 \pm 84.39)</td>
<td>(29.31 \pm 13.46)</td>
<td>(23.10 \pm 7.93)</td>
</tr>
<tr>
<td>2000 ((3/2))</td>
<td>(204.9 \pm 118.8)</td>
<td>(93.9 \pm 72.30)</td>
<td>(21.48 \pm 5.49)</td>
<td>(18.08 \pm 13.82)</td>
</tr>
</tbody>
</table>

AUC, area under the plasma concentration curve; \(C_{\text{max}}\), maximum plasma concentration; \(C_{\text{min}}\), minimum plasma concentration.
PTK/ZK was rapidly absorbed following oral administration, with a peak plasma concentration ($C_{\text{max}}$) reached in 1 to 2.5 h for all dose groups. At steady state, which was achieved by day 15, the systemic exposure (AUC) was ~30% lower than the exposure following a single dose for all doses >150 mg/day. A representative PTK/ZK plasma concentration profile on days 1, 15 and 28 from a patient who received 1200 mg/day PTK/ZK dose is shown in Figure 2. Metabolism via the CYP3A4 isoenzyme is the major elimination pathway for PTK/ZK, and autoinduction of this enzyme is the likely explanation for the observed decrease in AUC from day 1 to day 15. No further reduction in AUC was observed after day 15 and up to day 28. The half-life was ~3–6 h for each dose group on day 2 and EC1. PTK/ZK exposure appeared to be dose proportional up to 1000 mg/day at both day 2 and EC1; however, the exposure was less than dose-proportional at doses >1000 mg/day (Figure 2).

Biomarker assessment and statistical analysis

The mean and standard deviation for plasma VEGF-A and bFGF were 64.31 ± 48.11 and 14.73 ± 17.03 pg/ml, respectively. The mean and standard deviation for serum sTIE-2 and sE-SEL were 79.14 ± 27.51 and 62.57 ± 30.56 pg/ml, respectively. Differences in disease stage and tumor volume may be explanations for variability in the baseline level of plasma and serum markers; however, no significant differences were observed in baseline measurements of VEGF-A and bFGF between progressors and non-progressors, thereby excluding bias.

Changes in plasma VEGF-A and bFGF levels were observed with PTK/ZK treatment in the first cycle of treatment, and were prominent for patients who received ≥1000 mg dose (Figure 3). Plasma levels of VEGF-A and bFGF showed an exposure- and concentration-dependent increase in the first cycle of treatment, followed by a decline during the second cycle of treatment. The mean change from baseline VEGF-A was significantly correlated with PTK/ZK AUC at EC1 ($P=0.049$), with a positive trend on day 1 ($P=0.063$). The correlation between mean percentage of baseline VEGF-A and maximum PTK/ZK concentration also showed a positive trend on day 1 ($P=0.086$) and EC1 ($P=0.094$). The correlations

Figure 3. Percentage change from baseline for soluble markers across 56 days of PTK787/ZK 222584 (PTK/ZK) treatment for patients who received at least 1000 mg/day.

Figure 4. $E_{\text{max}}$ model characterizing the mean change in plasma VEGF-A concentration in first cycle of PTK787/ZK222584 (PTK/ZK) treatment (measured as percentage of baseline plasma VEGF-A) versus (top) plasma PTK/ZK $C_{\text{max}}$ on day 1 and (bottom) plasma PTK/ZK AUC$_{0-\infty}$ on day 1. One patient with high percentage of baseline VEGF-A not shown in plot, but included in regression. $E_{\text{max}}$, maximum effect; VEGF, vascular endothelial growth factor; $C_{\text{max}}$, maximum plasma concentration; AUC$_{0-\infty}$, area under the plasma concentration curve from time 0 to infinity.
between PTK/ZK exposure and concentration versus mean percentage of baseline bFGF did not reach statistical significance at any time point (data not shown). No changes in sTIE-2 and sE-SEL were observed at any dose level with PTK/ZK treatment, and therefore no subsequent analyses were performed.

The relationship between a drug and the biological response is usually complex, and in most cases can be described by a saturable system, such as the $E_{\text{max}}$ model. The $E_{\text{max}}$ model was used to characterize the relationship between mean percentage of baseline plasma VEGF-A versus AUC and $C_{\text{max}}$ (Figure 4). Although plasma bFGF also demonstrated a dose-dependent increase in the first cycle of treatment, the $E_{\text{max}}$ model did not appear to adequately characterize the relationship between mean percentage of baseline plasma bFGF versus AUC and $C_{\text{max}}$, and therefore no regression line was shown.

Figure 4 shows that most non-progressors achieved a PTK/ZK AUC of 100 h $\times$ $\mu$M and $C_{\text{max}}$ of 15 $\mu$M on day 1, both of which correspond to a mean change from baseline VEGF-A of 150%. These results suggest that a mean change from baseline VEGF-A of 150% correlated with non-progressive disease, and that this pharmacodynamic effect requires a dose $\geq$1000 mg/day.

Correspondingly, at 1000 mg/day PTK/ZK, the lower limit of the standard deviation at day 1 lies close to an AUC of 100 h $\times$ $\mu$M and $C_{\text{max}}$ of 15 $\mu$M. At this AUC and $C_{\text{max}}$, the mean change from baseline bFGF is ~400%.

A logistic model revealed a statistically significant relationship between change in plasma VEGF-A in the first cycle of PTK/ZK treatment and disease status ($P = 0.027$). This suggests that the change in plasma VEGF-A in the first cycle of PTK/ZK treatment is a good indicator of clinical outcome; i.e. a mean percentage of baseline VEGF-A of 150% correlates with a 50% probability of achieving non-progressive disease. In Figure 5, a plot of the best change in tumor size in the first two cycles of PTK/ZK treatment versus mean percentage of baseline VEGF-A in the first cycle of treatment supports this and the previous findings that most non-progressors achieved a 150% change from baseline plasma VEGF-A in the first cycle of treatment. Non-progressors achieved up to 40% tumor regression in the first or second cycle of PTK/ZK treatment. The change in plasma bFGF did not show statistical significance with disease status ($P = 0.633$) and is therefore not a good indicator of non-progressive disease.

An exploratory analysis (Figure 6) showed a trend toward increased tumor size in patients with higher baseline plasma VEGF-A levels, suggesting that larger tumor size upregulates the expression of VEGF-A as a regulatory control mechanism to promote neovascularization.

**Discussion**

Early clinical trials with molecular targeting agents should include the measurement of biomarkers to assess the pharmacodynamic effect of the molecular target and the relevant downstream components that may correlate with pharmacologic response. These two phase I/II studies are the first to demonstrate that the biological activity of an antiangiogenic agent may be assessed by the measurement of plasma VEGF-A and bFGF concentrations in patients with advanced CRC.

Several studies have implicated the VEGF pathway in human colon cancer angiogenesis [11, 12]. Further studies have assessed the role of VEGF signaling in the angiogenesis, metastasis and proliferation of human colon cancer [13, 14]. These studies support the hypothesis that VEGF signaling is an important angiogenic factor in colon cancer, and indicate that vessel count and the expression of VEGF may be useful in predicting metastasis from colon cancer [13]. Other studies have examined the regulation of VEGF secretion by colon cancer cells in vitro and showed that alteration of VEGF expression by colon cancer cells may affect the proliferative activity of the target endothelial cells [14].
Intuitively, one would expect that if PTK/ZK treatment is effective, a decrease in VEGF-A expression would be observed. These results, however, at early time points show a positive dose relationship with percentage change from baseline VEGF-A and bFGF, with a subsequent decline in VEGF levels as treatment continues. Similar findings have been observed in a mouse tumor model. In tumor-bearing mice, plasma VEGF-A levels were correlated with tumor burden. However, in comparison with vehicle-treated mice, plasma concentrations of VEGF-A in mice treated with 50 mg/kg PTK/ZK were found to increase after 1 day of treatment, with a subsequent decrease of plasma VEGF after 8 days of treatment. This decline in VEGF-A levels correlated with a significant decrease in tumor burden in the PTK/ZK-treated mice. The acute rise in plasma VEGF-A and bFGF concentrations observed in the animal studies and in the clinical trials described herein would be consistent with an initial increased expression of VEGF-A and bFGF by tumor cells in response to hypoxia induced by the reduction in tumor vascular permeability and vascularization induced by PTK/ZK treatment, with concomitant decreases in VEGF-A expression as the size of the tumor stabilizes or regresses. Previously reported results of DCE-MRI analysis in patients with CRC [15], as well as in animal studies [16, 17], have clearly shown an early reduction in tumor vascular permeability and vascularization with PTK/ZK treatment that significantly correlates with subsequent clinical response.

In these studies, non-progressive disease was associated with PTK/ZK doses ≥1000 mg/day. At 1000 mg/day PTK/ZK, a mean 150% increase from baseline VEGF-A levels was observed, and this may be considered a threshold that is associated with non-progressive disease. The percentage change from baseline VEGF-A in cycle 1 of treatment was significantly correlated with clinical outcome at the end of cycle 2, demonstrating the early clinical activity of PTK/ZK and the utility of plasma VEGF-A levels as a biomarker in helping to establish the optimal biological dose for PTK/ZK in this patient population. It can be hypothesized that the decline in plasma VEGF-A seen in the higher doses (≥1000 mg) after the first cycle may be due to the regressing tumor size with PTK/ZK treatment, but other factors may be involved.

This may indicate that an early rise in plasma VEGF-A is desirable and is suggestive of biological activity with PTK/ZK treatment; however, a decline in plasma VEGF-A is generally the real indicator of tumor regression. Changes from baseline VEGF-A appear to be a better indicator of clinical outcome than bFGF, suggesting that blockage of VEGF receptor activity by PTK/ZK has more impact in the mechanism by which angiogenesis is promoted. Changes in the endothelial cell-associated molecules sTIE-2 and sE-SEL were not observed over ~2 months of treatment with PTK/ZK, suggesting that these molecules have limited application as biomarkers for assessment of therapy.

These studies have shown that the plasma concentrations of VEGF-A and bFGF may be useful as biomarkers to detect biological activity in patients with CRC under treatment with PTK/ZK, and may therefore be of value in defining the optimal biologically active dose for phase II/III studies.

These results correlate strongly with those reported by Morgan et al. [15] in patients with CRC treated with oral, once-daily PTK/ZK at doses ranging from 50 to 2000 mg/day. In that study, early changes in tumor vascularity and vascular permeability (Ki) were assessed by DCE-MRI as biomarkers of clinical activity and correlated with PTK/ZK pharmacokinetics and subsequent clinical outcome after 56 days of treatment. A 60% decrease in Ki was significantly correlated with non-progressive disease after two cycles of PTK/ZK treatment, which required a dose ≥1000 mg/day PTK/ZK to achieve [15]. Taken together, these biomarker studies support the conclusion that the optimal biological dose of PTK/ZK in patients with CRC will be ≥1000 mg/day. However, the biomarker data alone are not sufficient to determine the optimal biological dose for further clinical testing. That determination must incorporate changes in tumor size, safety and pharmacokinetic data from studies that are currently underway.

Acknowledgements
The authors wish to thank Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA, and Schering AG, Berlin, Germany for their support of this study.

References


