Published in final edited form as:

Eur J Cancer. 2011 May ; 47(7): 997–1005. doi:10.1016/j.ejca.2010.12.016.

Phase I Trial of Vandetanib and Bevacizumab Evaluating the VEGF and EGF Signal Transduction Pathways in Adults With Solid Tumours and Lymphomas

Shivaani Kummar^{a,b,*}, Martin E. Gutierrez^a, Alice Chen^b, Ismail B. Turkbey^a, Deborah Allen^a, Yvonne R. Horneffer^a, Lamin Juwara^c, Liang Cao^a, Yunkai Yu^a, Yeong Sang Kim^a, Jane Trepel^a, Helen Chen^b, Peter Choyke^a, Giovanni Melillo^c, Anthony J. Murgio^{a,b}, Jerry Collins^b, and James H. Doroshow^{a,b}

^a Center for Cancer Research, National Cancer Institute, 10 Center Drive, Bethesda, MD 20892, USA

^b Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD 20892, USA

^c Science Applications International Corporation-Frederick, Inc., National Cancer Institute at Frederick, Frederick, MD 21702, USA

Abstract

PURPOSE—Inhibition of epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) pathways may result in synergistic antitumour activity. We designed a phase I study to evaluate the combination of vandetanib, an investigational agent with activity against EGF receptor and VEGF receptor 2, and bevacizumab, a monoclonal antibody against VEGF.

EXPERIMENTAL DESIGN—Patients with advanced solid tumours and lymphomas were enrolled. Objectives were to determine the safety and maximum tolerated dose of the combination, characterise pharmacokinetics, measure angiogenic marker changes in blood, and assess tumour blood flow using dynamic contrast enhanced magnetic resonance imaging (DCE-MRI). Vandetanib was given orally once daily and bevacizumab intravenously once every 3 weeks in 21-day cycles utilizing a standard dose escalation design.

RESULTS—Fifteen patients were enrolled, and a total of 94 cycles of therapy were administered. No protocol-defined dose-limiting toxicities were observed; due to toxicities associated with chronic dosing, hypertension, proteinuria, diarrhea, and anorexia, dose escalation was stopped at the second dose level. We observed one partial response and one minor response; nine patients experienced stable disease. There were significant changes in plasma VEGF and placental-derived growth factor levels, and decreases in K^{trans} and k_{ep} were observed by DCE-MRI.

CONCLUSION—In this trial, we safely combined two targeted agents that cause dual blockade of the VEGF pathway, demonstrated preliminary evidence of clinical activity, and conducted

*Corresponding author: Tel.: +1 301 496 4291; fax: +1 301 496 0826. kummar@mail.nih.gov (S. Kummar).

Presented in part at the 45th Annual Meeting of the American Society of Clinical Oncology, Orlando, FL, May 29-June 2, 2009 and the 2010 Gastrointestinal Cancers Symposium, Orlando, FL, January 22-24 2010.

Conflict of interest statement: None declared.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

correlative studies demonstrating anti-angiogenic effect. The recommended phase II dose was established as vandetanib 200 mg daily and bevacizumab 7.5 mg/kg every 3 weeks.

Keywords

clinical trial; phase I; vandetanib; bevacizumab; VEGF inhibitor; EGF inhibitor; ZD6474

INTRODUCTION

Combining targeted anticancer agents is one approach to improving the therapeutic outcome for patients with cancer. Bevacizumab is a humanised anti vascular endothelial growth factor (VEGF) monoclonal antibody that prevents the interaction of VEGF with its receptors on the surface of endothelial cells.¹ It is approved by the US Food and Drug Administration for treatment of colorectal and lung cancer. Vandetanib is an investigational small molecule that inhibits VEGF receptor 2 (VEGFR2), epidermal growth factor receptor (EGFR), and RET (rearranged during transfection) receptor tyrosine kinases.^{2–4} Vandetanib is generally well tolerated as a single agent in patients at a dose of 300 mg/day.⁵ Because the epidermal growth factor (EGF) and VEGF pathways are aberrantly activated in several types of cancers, we hypothesised that inhibition of both pathways would result in synergistic antitumour activity.^{6, 7} In addition, dual blockade of the VEGF pathway by the two agents may provide an added therapeutic effect.^{8, 9}

We conducted a phase I dose-escalation study to determine the safety, toxicity, and maximum tolerated dose (MTD) of vandetanib in combination with bevacizumab in patients with advanced malignancies refractory to standard therapy. To assess the effects of the drug combination on angiogenesis, we measured VEGF and placental-derived growth factor (PlGF) in plasma, numbers of circulating endothelial progenitors (CEPs) and mature circulating endothelial cells (CECs), and changes in tumour vascular permeability using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) before and after drug administration.

PATIENTS AND METHODS

Eligibility Criteria

Patients (age ≥ 18 years) were eligible if they had pathologically confirmed metastatic malignancy for which there were no acceptable standard therapies; an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 (Karnofsky $\leq 60\%$); and adequate organ and marrow function defined as absolute neutrophil count $\geq 1,500/\mu\text{L}$, platelets $\geq 100,000/\mu\text{L}$, total bilirubin $\leq 1.5 \times$ the upper limit of normal (ULN), aspartate aminotransferase and/or alanine aminotransferase $< 2.5 \times$ ULN, creatinine $< 1.5 \times$ ULN, Urine Protein Creatinine (UPC) ratio of < 0.5 or 24-hour urine protein < 1000 mg. Prior anti-VEGF therapy was allowed.

Prior anticancer therapy or surgery must have been completed at least 4 weeks before starting the study drugs; toxicities from previous treatment were required to have recovered to eligibility levels. Patients were excluded if they had an uncontrolled intercurrent illness; had uncontrolled hypertension (defined as BP $> 150/90$ mm Hg despite therapy); were pregnant or lactating; had lung carcinoma of squamous cell histology; had hemoptysis within the past 3 months; QTc > 480 msec; were taking medications that might cause QTc prolongation; required therapeutic anticoagulation; or had received treatment for brain metastases within the past 3 months. Other exclusion criteria included a history of symptomatic congestive heart failure, significant vascular disease, unstable angina pectoris,

or myocardial infarction within 6 months of study entry, non-healing wounds, or uncontrolled cardiac arrhythmias.

This trial was conducted under a National Cancer Institute (NCI)-sponsored IND with institutional review board approval. The protocol design and conduct followed all applicable regulations, guidances, and local policies. [ClinicalTrials.gov Identifier: NCT00734890].

Trial Design

This was an open-label, single-arm phase I combination study of vandetanib and bevacizumab in patients with advanced malignancies. Bevacizumab was supplied by the Division of Cancer Treatment and Diagnosis, NCI under a Collaborative Research and Development Agreement with Genentech. Vandetanib was obtained under a separate collaborative agreement with AstraZeneca, Inc. Vandetanib was administered orally (PO) once daily, days 1 to 21, and bevacizumab intravenously (IV; over 90 minutes for the first dose and then over 30 to 60 minutes) on day 1 of every cycle; cycle length 21 days. The starting dose of vandetanib, 100 mg daily, was 33.3% of the recommended single-agent phase II dose of 300 mg,^{5, 10} and the starting dose of bevacizumab was 7.5 mg/kg every 3 weeks. Dose escalation of vandetanib (to 200 then 300 mg) was planned first, followed by dose escalation of bevacizumab (to 10 then 15 mg/kg) if tolerated.

A standard phase I dose escalation design was employed, whereby groups of three to six patients were treated at each dose level. Higher dose levels were not opened to accrual until the last patient in the previous cohort had completed two cycles.

Adverse events were graded according to NCI Common Toxicity Criteria version 3.0. Dose limiting toxicity (DLT) was defined as an adverse event that occurred in the first two cycles, was felt to be related to the study drug, and fulfilled one of the following criteria: grade 3 or greater non-haematologic toxicity (except for grade 3 hypertension controlled by oral medication, grade 3 nausea/vomiting and diarrhea without maximal symptomatic treatment, grade 3 creatinine and electrolyte abnormalities that corrected to grade 1 or baseline within 24 hours); grade 4 haematologic toxicity; or toxicity delaying treatment for more than 7 days.

Safety and Efficacy Evaluations

History, physical examination, and complete blood counts with differential and serum chemistries were performed at baseline and once a week on treatment. Blood pressure was measured weekly by a health care provider during the first two cycles, daily at home by the patients for the first 6 weeks, and then at each doctor's visit thereafter. To evaluate for study drug effect on the QTc interval, three 12-lead electrocardiograms were performed pre-dose, and the baseline QTc was calculated as the average of the QTc intervals on the three EKGs. Follow-up EKGs were performed on weeks 1, 2, 4, 8, and 12, and then every 3 months while on study. Radiographic evaluation was performed at baseline and every two cycles to assess tumour response based on the Response Evaluation Criteria in Solid Tumors version 1.0 (RECIST).¹¹

Pharmacokinetics of Vandetanib

Blood samples were collected in 7-mL tubes containing lithium heparin at pre-dose and on cycle 3 day 1 at 4, 6, 8, and 24 hours post-dose. Samples were centrifuged within 30 minutes of collection, and plasma was stored frozen at -70°C until analysis. Concentrations of vandetanib were determined in four patients on dose level 2 using solvent extraction followed by reverse phase high performance liquid chromatography with tandem mass

spectrometric detection. This method was performed at Bioanalytical Systems Inc (West Lafayette, Indiana) under its contract with AstraZeneca.

Vandetanib was detected using a PerkinElmer Sciex quadrupole mass spectrometer using a turbo ion spray source (multiple reaction monitoring in positive ion mode). Concentrations were determined using a calibration curve prepared over a range of 5 to 1000 ng/mL vandetanib in 100 μ L aliquots of heparinised human plasma. Two sets of calibrators were included in each analytical batch, one at the beginning and one at the end. Data acquisition was performed using Analyst version 1.4.1 software.

Pharmacodynamics

Measurement of Plasma Biomarkers—Blood (7 mL) was collected in EDTA-containing Vacutainer tubes at baseline and on day 8 of cycles 1, 2, and 4. After centrifugation, samples were aliquoted, immediately frozen, and stored at -80°C . VEGF, PlGF, basic fibroblast growth factor (bFGF), and soluble VEGFR1 (sVEGFR1) were measured using assay plates, protein standards, and Sector 2400 software from Meso-Scale Discovery (Gaithersburg, MD). Median values with interquartile ranges were determined using Prism (GraphPad, La Jolla, CA). Comparisons between time points were made using a paired Student's t-test.

Assessment of CECs and CEPs—Blood was collected in two 8-mL CPT cell preparation tubes with sodium citrate (BD, Franklin Lakes, NJ) at baseline, on day 2 of cycle 1 (24 hours after bevacizumab administration), and on day 1 of cycles 2 and 4 (at the end of bevacizumab infusion). Mononuclear cells were isolated and viably frozen (Appendix, online only). CEC and CEP analyses were performed using an LSRII flow cytometer (Becton Dickinson, Franklin Lakes, NJ); a minimum of 1×10^5 cells were acquired for each analyses. Data were analysed using FlowJo software (Tree Star, Ashland, OR). CECs were defined as negative for the hematopoietic marker CD45, positive for the endothelial markers CD31 and CD146, and negative for the progenitor marker CD133. CEPs were defined as the CD45 $-$ /CD31 $+$ /CD146 $-$ /CD133 $+$ population. Viability was defined by Hoechst 33258 negativity.

DCE-MRI—DCE-MRI scans and analysis were performed using standard methods as described in the Appendix (online only). Imaging was performed at baseline and after two cycles of treatment.

RESULTS

Patients

Patient characteristics are detailed in Table 1. Fifteen patients were accrued; 3 to dose level 1 (vandetanib 100 mg PO daily and bevacizumab 7.5 mg/kg IV every 3 weeks) and 12 to dose level 2 (vandetanib 200 mg PO daily and bevacizumab 7.5 mg/kg IV every 3 weeks). A total of 94 cycles of therapy were administered, and 9 of the patients received more than 4 cycles of therapy. Five had received bevacizumab in combination with chemotherapy as part of prior therapies.

Toxicity

Hypertension and rise in creatinine were observed with continued dosing beyond cycle 1 in this trial. All patients on dose level 1 experienced grade ≥ 2 hypertension, and one patient had a grade 1 rise in creatinine. One patient with metastatic melanoma in dose level 1 developed grade 3 neutropenia and grade 4 thrombocytopenia during cycle 5 week 2; there was no evidence of associated hemolysis, fever, or renal dysfunction. Platelet counts

dropped to 1,000/ μ L with associated epistaxis. This patient was treated conservatively with transfusions and gradually recovered. There were no protocol defined dose limiting toxicities in the first three patients at dose level 2; however, due to the toxicities observed with continued therapy, we stopped dose escalation and expanded dose level 2 following discussions with the study sponsor and the institutional review board. Of the first six patients enrolled on dose level 2, three experienced grade ≥ 2 hypertension. There were also four instances of grade 1 rise in creatinine and two instances of grade 2 rise in creatinine after the first cycle. Other common adverse events grade ≥ 2 were rash, proteinuria, and prolonged QTc (Table 2). All toxicities were manageable by either holding the dose of study drugs for a few days or optimizing antihypertensive medications.

Antitumour Activity

Thirteen patients had measurable disease and were evaluable for response (Fig. 1). We observed one partial response (duration 8 cycles) in a patient with metastatic jejunal adenocarcinoma and one minor response in a patient with non-small cell lung cancer (adenocarcinoma). Nine patients had stable disease, four of which were for eight cycles or longer (pancreatic adenocarcinoma (1), colorectal cancer (2), peritoneal mesothelioma (1)). Interestingly, the patients with peritoneal mesothelioma and colorectal cancer had received prior bevacizumab, and the patient with adenocarcinoma of the lung with a minor response had received prior sorafenib. The patient with pancreatic cancer had prolonged disease stabilization (20 cycles) of the target lesions. While on study, this patient was noted to have an ovarian cyst that was followed and gradually grew. Resection of this cyst later revealed pancreatic cancer.

Pharmacokinetics

The addition of bevacizumab did not appear to alter the pharmacokinetics of vandetanib. Steady-state vandetanib levels were achieved in four patients on dose level 2 (200 mg) evaluated on cycle 3 day 1, as indicated by similar trough concentrations at 0 and 24 hours. This is consistent with that previously reported in single-agent studies of vandetanib.^{5, 10}

Pharmacodynamics

Measurement of Plasma Biomarkers—Significant increases in median VEGF and PlGF (placental-derived growth factor) levels were observed at all post-dose time points ($P < .001$; Appendix Table A1, online only). The fold increase from baseline was greater in the levels of VEGF than PlGF. In contrast, only a transient decrease in median sVEGFR1 was observed on day 8 of cycle 1 ($P = .01$) while no change in bFGF levels were observed at any time point (Appendix Table A1, online only).

Assessment of CECs and CEPs—CECs and CEPs were analysed in peripheral blood mononuclear cell (PBMC) samples from 13 patients. In all samples, $> 80\%$ of the CEPs were viable while $> 70\%$ of the CECs were apoptotic as determined by Hoechst staining (data not shown). Total CEC numbers increased compared to baseline in 6 of 12 patients at cycle 2 day 1 (Fig. 2A), while CEP levels decreased in 8 of 12 patients at the same time point (Fig. 2B). Consistent with an anti-angiogenic effect, 4 of 12 patients at cycle 2 day 1 had increased CEC levels and decreased CEP levels. A correlation between CEC and CEP levels and clinical response was not observed, potentially due to the small number of total patients and responders.

DCE-MRI—Fifteen patients had DCE-MRI analysis at baseline, and target lesions were localized in lung (5 patients), liver (3 patients), mesentery (2 patients), adrenal gland (1 patient), axilla (1 patient), paratracheal space (1 patient), retroperitoneum (1 patient), and

chest wall (1 patient). All patients had follow-up DCE-MRI scans after treatment; however, scans from three patients were excluded due to suboptimal contrast injection timing. Scans of the 12 evaluable patients were analysed with a two-compartment model to derive K^{trans} , the forward contrast transfer rate, and k_{ep} , the reverse contrast transfer rate values. Decreases in both K^{trans} and k_{ep} were seen in six patients (Fig. 3A and 3B).

DISCUSSION

In this study we safely administered bevacizumab and vandetanib with manageable side effects and observed clinical activity. Although our patients were heavily pre-treated, we observed one partial response in a patient with metastatic jejunal adenocarcinoma (five prior lines of therapy) and one minor response in a patient with adenocarcinoma of the lung (two prior lines of therapy). There were no toxicities that met the predefined criteria for DLT; however, because of the toxicities observed with continued therapy, the serious toxicity observed in one patient with chronic dosing, and the clinical activity already observed at the doses tested, the decision was made to stop dose escalation. The recommended phase II dose for this combination is vandetanib 200 mg PO daily and bevacizumab 7.5 mg/kg IV every 3 weeks.

The important considerations raised by this study are the toxicities associated with dual blockade of the VEGF pathway, toxicities that were both acute and related to the duration of drug exposure, and that might have been affected by prior exposure to anti-angiogenic therapy. Thus, the conventional phase I trial design, originally developed for cytotoxic chemotherapies to determine dose escalation and safety from first-cycle DLT, was inadequate. In this study, toxicities such as hypertension and proteinuria increased in grade with continued treatment. Eight of the 15 patients had baseline hypertension that worsened on study, and 5 of these patients had worsening of hypertension within the first 3 cycles of therapy; all required further dose optimization and/or addition of another antihypertensive with higher cycle numbers. A similar trend was observed for proteinuria, with grade 1 or 2 proteinuria developing with continued therapy. Thus, the highest grade of toxicity was not necessarily observed in cycle 1 and would not have affected determination of the MTD as defined by conventional criteria. Anticipating this consideration and given that steady-state levels of vandetanib are achieved after at least 4 weeks of dosing,^{5, 10} we defined DLT over two cycles so that toxicity could be established more accurately.

The common toxicities observed on this study, namely hypertension, rise in creatinine, and proteinuria have been observed with other anti-angiogenic therapies and were therefore expected. An increased incidence of hypertension (ranging from 2.7% to 36%, depending on dose) has been reported associated with the use of bevacizumab. In a meta-analysis of 1,850 patients treated with bevacizumab, the incidence of proteinuria (transient albuminuria to nephrotic-range proteinuria in 1% to 1.8% of the cases) was reported to be from 21% to over 60% depending on the dose.¹² The underlying etiology of proteinuria has been shown to be related to the development of thrombotic microangiopathy and may be a consequence of the reduction of VEGF in podocytes, as evidenced by deletion of the VEGF gene from podocytes in a murine model, which resulted in proteinuria with features of thrombotic microangiopathy.¹³

Dual blockade of the VEGF pathway has been poorly tolerated. In the recent report of the phase I trial of bevacizumab with sunitinib in patients with metastatic renal cell carcinoma, hypertension was observed in 92% of the treated patients, with several cases of microangiopathic hemolytic anaemia noted based on clinical symptoms and retrospective review of peripheral smears in patients with thrombocytopenia.¹⁴ One patient developed grade 4 thrombocytopenia with no evidence of schistocytosis or ongoing hemolysis;

however, there was concern about continuing to escalate doses in subsequent patients with the resultant potential increased risk of serious toxicities. Our results indicate adaptation of the vandetanib dose to 200 mg/day from the 300 mg/day single-agent dose is safe when given in combination with bevacizumab.

This trial also highlights the importance of evaluating how best to safely and effectively combine molecularly targeted therapies to optimise clinical benefit. An appreciable number of the combinations tested to date in early-phase clinical trials have been either empirically derived or based on minimal preclinical (especially *in vivo*) data for both potential antitumour effects and toxicities (on- and off-target). Much more needs to be done to understand the underlying biology of the targets and the interactions of potential inhibitors prior to the initiation of molecularly targeted combinatorial studies, as exemplified by the absence of clinical benefit observed when cetuximab was added to bevacizumab-containing regimens in patients with colorectal cancer. Even though there were early-phase data supporting the addition of anti-EGFR therapy to bevacizumab and chemotherapy,¹⁵ outcomes such as progression-free survival were actually worse with the addition of cetuximab or panitumumab.^{16, 17}

We demonstrated significant increases in plasma VEGF and PlGF levels in this study, supporting the anti-angiogenic effect of the combination of vandetanib and bevacizumab. Changes in CECs and CEPs were consistent with observations in mouse studies with vandetanib.¹⁸ CEPs have been shown to infiltrate human tumours and give rise to tumour neovasculation,^{19, 20} whereas mature CECs derive from mature vasculature. Inhibition of the VEGF pathway can have different effects on mature CECs and CEPs.^{18, 21–23} VEGF pathway inhibitors inhibit bone marrow-derived CEPs mobilised by VEGF²⁴ and can trigger an increase in mature CECs, reflecting sloughing of fragile mature endothelium from tumour vasculature and potentially other sources.^{18, 25} In mouse model studies, these changes were associated with decreases in tumour microvessel density and occurred before reductions in tumour volume.¹⁸

In this trial, we were able to safely combine two targeted agents that cause dual blockade of the VEGF pathway, and demonstrate preliminary evidence of clinical activity of the regimen. However, further development of this combination should be viewed in light of the experience emerging from clinical trials of combined blockade of the anti-VEGF pathway, its potential for clinical benefit and significant toxicities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Center for Cancer Research and the Division of Cancer Treatment and Diagnosis of the National Cancer Institute, National Institutes of Health. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

We thank Gina Uhlenbrauck and Yvonne A. Evrard, SAIC-Frederick, Inc., for editorial assistance in the preparation of this manuscript. We gratefully acknowledge Bill Telford and Veena Kapoor for support and use of the NCI/ETIB Flow Cytometry Core Facility.

References

1. Ranieri G, Patruno R, Ruggieri E, Montemurro S, Valerio P, Ribatti D. Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. *Curr Med Chem*. 2006; 13:1845–57. [PubMed: 16842197]
2. Carlomagno F, Vitagliano D, Guida T, et al. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. *Cancer Res*. 2002; 62:7284–90. [PubMed: 12499271]
3. Wedge SR, Ogilvie DJ, Dukes M, et al. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. *Cancer Res*. 2002; 62:4645–55. [PubMed: 12183421]
4. Ciardiello F, Caputo R, Damiano V, et al. Antitumor effects of ZD6474, a small molecule vascular endothelial growth factor receptor tyrosine kinase inhibitor, with additional activity against epidermal growth factor receptor tyrosine kinase. *Clin Cancer Res*. 2003; 9:1546–56. [PubMed: 12684431]
5. Holden SN, Eckhardt SG, Bassler R, et al. Clinical evaluation of ZD6474, an orally active inhibitor of VEGF and EGFR receptor signaling, in patients with solid, malignant tumors. *Ann Oncol*. 2005; 16:1391–97. [PubMed: 15905307]
6. Pennell NA, Lynch TJ Jr. Combined inhibition of the VEGFR and EGFR signaling pathways in the treatment of NSCLC. *Oncologist*. 2009; 14:399–411. [PubMed: 19357226]
7. Tonra JR, Deevi DS, Corcoran E, et al. Synergistic antitumor effects of combined epidermal growth factor receptor and vascular endothelial growth factor receptor-2 targeted therapy. *Clin Cancer Res*. 2006; 12:2197–207. [PubMed: 16609035]
8. Matar P, Rojo F, Cassia R, et al. Combined epidermal growth factor receptor targeting with the tyrosine kinase inhibitor gefitinib (ZD1839) and the monoclonal antibody cetuximab (IMC-C225): superiority over single-agent receptor targeting. *Clin Cancer Res*. 2004; 10:6487–501. [PubMed: 15475436]
9. Huang S, Armstrong EA, Benavente S, Chinnaiyan P, Harari PM. Dual-agent molecular targeting of the epidermal growth factor receptor (EGFR): combining anti-EGFR antibody with tyrosine kinase inhibitor. *Cancer Res*. 2004; 64:5355–62. [PubMed: 15289342]
10. Tamura T, Minami H, Yamada Y, et al. A phase I dose-escalation study of ZD6474 in Japanese patients with solid, malignant tumors. *J Thorac Oncol*. 2006; 1:1002–09. [PubMed: 17409986]
11. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst*. 2000; 92:205–16. [PubMed: 10655437]
12. Zhu X, Wu S, Dahut WL, Parikh CR. Risks of proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis. *Am J Kidney Dis*. 2007; 49:186–93. [PubMed: 17261421]
13. Eremina V, Jefferson JA, Kowalewska J, et al. VEGF inhibition and renal thrombotic microangiopathy. *N Engl J Med*. 2008; 358:1129–36. [PubMed: 18337603]
14. Feldman DR, Baum MS, Ginsberg MS, et al. Phase I trial of bevacizumab plus escalated doses of sunitinib in patients with metastatic renal cell carcinoma. *J Clin Oncol*. 2009; 27:1432–39. [PubMed: 19224847]
15. Saltz LB, Lenz HJ, Kindler HL, et al. Randomized phase II trial of cetuximab, bevacizumab, and irinotecan compared with cetuximab and bevacizumab alone in irinotecan-refractory colorectal cancer: the BOND-2 study. *J Clin Oncol*. 2007; 25:4557–61. [PubMed: 17876013]
16. Tol J, Koopman M, Cats A, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med*. 2009; 360:563–72. [PubMed: 19196673]
17. Hecht JR, Mitchell E, Chidiac T, et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. *J Clin Oncol*. 2009; 27:672–80. [PubMed: 19114685]
18. Beaudry P, Force J, Naumov GN, et al. Differential effects of vascular endothelial growth factor receptor-2 inhibitor ZD6474 on circulating endothelial progenitors and mature circulating

- endothelial cells: implications for use as a surrogate marker of antiangiogenic activity. *Clin Cancer Res.* 2005; 11:3514–22. [PubMed: 15867254]
19. Peters BA, Diaz LA, Polyak K, et al. Contribution of bone marrow-derived endothelial cells to human tumor vasculature. *Nat Med.* 2005; 11:261–62. [PubMed: 15723071]
20. Ahn GO, Brown JM. Role of endothelial progenitors and other bone marrow-derived cells in the development of the tumor vasculature. *Angiogenesis.* 2009; 12:159–64. [PubMed: 19221886]
21. Schuch G, Heymach JV, Nomi M, et al. Endostatin inhibits the vascular endothelial growth factor-induced mobilization of endothelial progenitor cells. *Cancer Res.* 2003; 63:8345–50. [PubMed: 14678995]
22. Willett CG, Boucher Y, Duda DG, et al. Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. *J Clin Oncol.* 2005; 23:8136–9. [PubMed: 16258121]
23. Duda DG, Cohen KS, Ancukiewicz M, et al. A comparative study of circulating endothelial cells (CECs) and circulating progenitor cells (CPCs) kinetics in four multidisciplinary phase II studies of antiangiogenic agents. *J Clin Oncol.* 2008; 26:3544. (abstract).
24. Capillo M, Mancuso P, Gobbi A, et al. Continuous infusion of endostatin inhibits differentiation, mobilization, and clonogenic potential of endothelial cell progenitors. *Clin Cancer Res.* 2003; 9:377–82. [PubMed: 12538491]
25. Monestiroli S, Mancuso P, Burlini A, et al. Kinetics and viability of circulating endothelial cells as surrogate angiogenesis marker in an animal model of human lymphoma. *Cancer Res.* 2001; 61:4341–44. [PubMed: 11389057]
26. Kety SS. The theory and applications of the exchange of inert gas at the lungs and tissues. *Pharmacol Rev.* 1951; 3:1–41. [PubMed: 14833874]

Fig. 1. Changes in tumour size from baseline assessed according to Response Evaluation Criteria in Solid Tumors (n = 13). One patient had nonmeasurable disease, and one patient was not evaluable for response (declined treatment after cycle 1).

Fig. 2.

(A) Numbers of circulating endothelial cells (CECs) $\times 10^{-3}$ and (B) circulating endothelial progenitors (CEPs) per 10^6 PBMCs were determined pre- and post-treatment by 7-parameter flow cytometric analysis. No cycle 2 day 1 sample was available for patient 10, and no cycle 4 day 1 sample was available for patients 5 and 9 through 15. Symbols indicate patients with prolonged disease stabilization (*), minor response (†), or partial response (‡).

Fig. 3.

Changes in tumour vascular permeability were assessed using DCE-MRI; parameters were determined before and after treatment. Quantitative analyses of (A) K^{trans} , the forward contrast transfer rate, (B) k_{ep} , the reverse contrast transfer rate were derived using a curve-fitting general kinetic model (GKM) algorithm. Symbols indicate patients with prolonged disease stabilization (*), minor response (†), or partial response (‡).

Table 1

Patient characteristics

Characteristic	No. of patients
Evaluable patients	15
Male	8
Female	7
Age, years	
Median	58
Range	35–75
ECOG performance status	
0	2
1	12
2	1
Tumor types	
Colorectal	4
Lung	3
Pancreas	2
Melanoma	1
Peritoneal mesothelioma	1
Non-Hodgkin's lymphoma	1
Small bowel	1
Adenocarcinoma	
Chondrosarcoma	1
Carcinoid	1
No. of prior lines of therapy	
Median	3
Range	1–9

ECOG, Eastern Cooperative Oncology Group.

Table 2

Grade of adverse events per patient, by cycle, felt to be at least possibly related to study drug combination

Patient	Prior anti-VEGF therapy	History of HTN	Adverse event	Grade of adverse event by cycle																
				1	2	3	4	5	6	7	8	12	15	16	20	22				
1	N	Y	HTN		2							3								
			Proteinuria		1				2											
			Weight loss											1		2				
			Prolonged QTc													2				
2 ^a	Y (sorafenib)	Y	HTN			3														
			Anorexia		1		2													
			Weight loss			1														
			Hand-foot syndrome					2												
3	N	Y	HTN			3	3	3												
			Leucopenia						3											
			Thrombo-cytopenia						4											
			Neutropenia						3											
			Lower GI hemorrhage						3											
4 ^b	Y (bevacizumab)	Y	HTN		3	3	3	3	3											
			Proteinuria						2											
			Elevated creatinine		1		3													
5	N	Y	Prolonged QTc		2															
			Rash				3													
			Neutropenia				2													
			Leucopenia				2													
6	N	N	Rash		2															
			HTN			1	2													
			Diarrhea			2	2													
			Prolonged QTc					2		2										
			Proteinuria						1											

Patient	Prior anti-VEGF therapy	History of HTN	Adverse event	Grade of adverse event by cycle																		
				1	2	3	4	5	6	7	8	12	15	16	20	22						
Weight loss				1 2																		
7	Y (bevacizumab)	Y	HTN	3																		
			Proteinuria	1 2																		
			Elevated creatinine	1 1 1 1																		
8 ^b	Y (bevacizumab)	Y	HTN	3	3	3	3	3														
			Proteinuria	1 2 2																		
			Elevated creatinine	1 2 2																		
			Anorexia	1 5																		
9	Y (bevacizumab)	N	Lower GI hemorrhage	2																		
			HTN	1																		
			Proteinuria	2																		
10	N	N	HTN	1																		
			Proteinuria	2																		
11	N	N	Proteinuria	1 2																		
			Pancreatitis	3																		
			Lymphopenia	3																		
12	Y (sorafenib)	N	HTN	2 3																		
			Anorexia	2																		
			Weight loss	1																		
13 ^b	Y (bevacizumab)	Y	HTN	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3			
			Proteinuria	2																		
			Anorexia	1																		
Weight loss				1																		
Rash				2																		
14	N	N																				

Patient	Prior anti-VEGF therapy	History of HTN	Adverse event	Grade of adverse event by cycle															
				1	2	3	4	5	6	7	8	12	15	16	20	22			
15	N	N	Proteinuria		1														

HTN, hypertension; GI, gastrointestinal.

^a Patient with adenocarcinoma of the lung and a minor response withdrew from the study due to chronic anorexia.

^b Antihypertensives had to be adjusted and doses increased or medications changed in later cycles to optimise control.