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# Phase I trial of vandetanib and bevacizumab evaluating the VEGF and EGF signal transduction pathways in adults with solid tumours and lymphomas ☆,☆☆

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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 in every 3 weeks in 21-day cycles utilising 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, diarrhoea and anorexia, dose escalation was stopped at the second dose level. We observed one partial response and one minor response; 9 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

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conducted 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.

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## 1. Introduction

Combining targeted anticancer agents is one approach to improve 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.

## 2. Patients and methods

### 2.1. Eligibility criteria

Patients (age $\geqslant$ 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 $\leqslant 2$ (Karnofsky $\geqslant$ 60%); and adequate organ and marrow function defined as absolute neutrophil count $\geqslant$ 1500/μl, platelets $\geqslant$ 100,000/μl, total bilirubin $\leqslant 1.5\times$ 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-h 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 haemoptysis within the past 3 months; QTc > 480 ms; 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).

### 2.2. 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 1–21, and bevacizumab intravenously (IV; over 90 min for the first dose and then over 30–60 min) 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 3–6 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 2 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 2 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 diarrhoea without maximal symptomatic treatment, grade 3 creatinine and electrolyte abnormalities that corrected to grade 1 or baseline within 24 h); grade 4 haematologic toxicity; or toxicity delaying treatment for more than 7 days.

### 2.3. 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 2 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 2 cycles to assess tumour response based on the Response Evaluation Criteria in Solid Tumors version 1.0 (RECIST).[11]

### 2.4. 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 h post-dose. Samples were centrifuged within 30 min of collection, and plasma was stored frozen at −70 °C until analysis. Concentrations of vandetanib were determined in 4 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 5–1000 ng/ml vandetanib in 100 μl 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.

### 2.5. Pharmacodynamics

#### 2.5.1. 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.

#### 2.5.2. 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 h 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 $1 \times 10^5$ 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.

#### 2.5.3. 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 2 cycles of treatment.

## 3. Results

### 3.1. 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.

### 3.2. 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 $\geqslant 2$ hypertension, and 1 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 haemolysis, fever, or renal dysfunction. Platelet counts dropped to 1000/μl with associated epistaxis. This patient was treated conservatively with transfusions and gradually recovered. There were no protocol-defined dose-limiting toxicities in the first 3 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 6 patients enrolled on dose level 2, three experienced grade $\geqslant 2$ 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 $\geqslant 2$ 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 optimising antihypertensive medications.

### 3.3. 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 8 cycles or longer (pancreatic adenocarcinoma (1), colorectal cancer (2) and peritoneal

**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 |
| Tumour 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.

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 stabilisation (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.

### 3.4. *Pharmacokinetics*

The addition of bevacizumab did not appear to alter the pharmacokinetics of vandetanib. Steady-state vandetanib levels were achieved in 4 patients on dose level 2 (200 mg) evaluated on cycle 3 day 1, as indicated by similar trough concentrations at 0 and 24 h. This is consistent with that previously reported in single-agent studies of vandetanib.[5,10]

### 3.5. *Pharmacodynamics*

#### 3.5.1. *Measurement of plasma biomarkers*

Significant increases in median VEGF and PIGF (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 PIGF. 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).

#### 3.5.2. *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.

#### 3.5.3. *DCE-MRI*

Fifteen patients had DCE-MRI analysis at baseline, and target lesions were localised 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 3 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 6 patients (Fig. 3A and B).

## 4. 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 1 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 optimisation and/or addition of another antihypertensive with higher cycle numbers. A similar trend was observed for proteinuria, with grade 1 or 2 protein-

**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 haemorrhage | | | | | 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 | | | | | | | | | | | |
| | | | Diarrhoea | | 2 | 2 | | | | | | | | | | |
| | | | Prolonged QTc | | | | 2 | | | 2 | | | | | | |
| | | | Proteinuria | | | 1 | | | | | | | | | | |
| | | | Weight loss | | | | 1 | | | | 2 | | | | | |
| 7 | Y (bevacizumab) | Y | HTN | | 3 | | | | | | | | | | | |
| | | | Proteinuria | | | | | | 1 | | 2 | | | | | |
| | | | Elevated creatinine | | 1 | 1 | 1 | 1 | 1 | | | | | | | |
| 8[b] | Y(bevacizumab) | Y | HTN | 3 | 3 | | 3 | | | | | 3 | | | | |
| | | | Proteinuria | | 1 | | | | 2 | | 2 | | | | | |
| | | | Elevated creatinine | 1 | 2 | | | | | | | 2 | | | | |
| | | | Anorexia | | 1 | | | | 5 | | | | | | | |
| 9 | Y(bevacizumab) | N | Lower GI haemorrhage | 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 | | | | | | |
| | | | Proteinuria | | | | | | 2 | | | | | | | |
| | | | Anorexia | | 1 | | | | | | | | | | | |
| | | | Weight loss | | | | | | 1 | | | | | | | |
| | | | Rash | 2 | | | | | | | | | | | | |
| 14 | N | N | | | | | | | | | | | | | | |
| 15 | N | N | Proteinuria | | 1 | | | | | | | | | | | |

HTN, hypertension and 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.

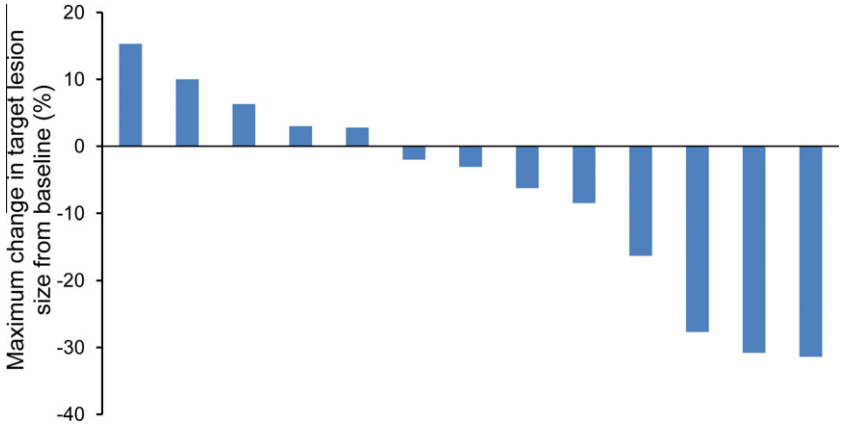


**Fig. 1 – Changes in tumour size from baseline assessed according to Response Evaluation Criteria in Solid Tumors ($n$ = 13). One patient had nonmeasurable disease and 1 patient was not evaluable for response (declined treatment after cycle 1).**

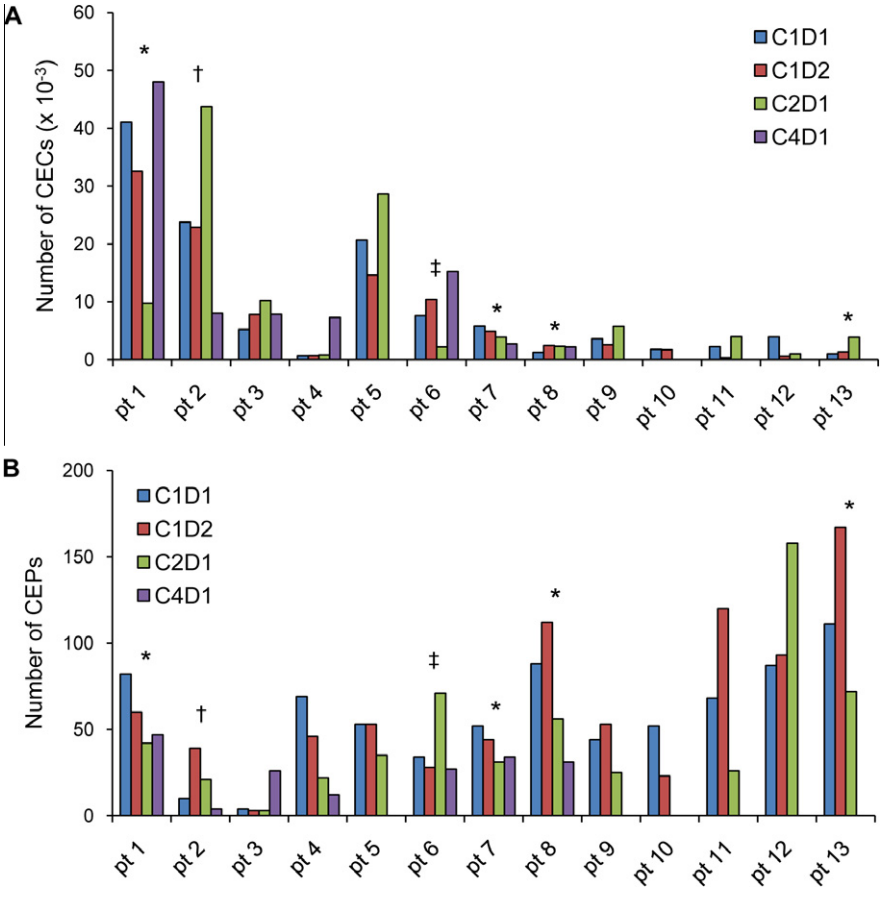


**Fig. 2 – (A) Numbers of circulating endothelial cells (CECs) $\times 10^{-3}$ and (B) circulating endothelial progenitors (CEPs) per $10^6$ PBMCs were determined pre- and post-treatment by seven-parameter flow cytometric analysis. No cycle 2 day 1 sample was available for patient 10 and no cycle 4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

uria 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 2 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

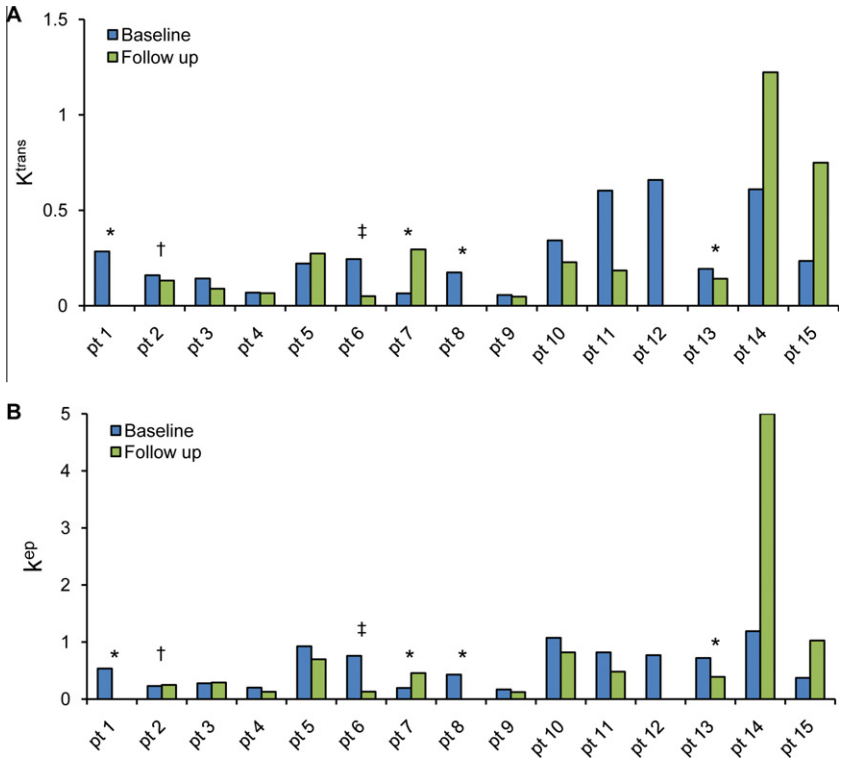


**Fig. 3 – Changes in tumour vascular permeability were assessed using DCE-MRI; parameters were determined before and after treatment. Quantitative analyses of (A) $K^{trans}$, the forward contrast transfer rate and (B) $k_{ep}$, the reverse contrast transfer rate were derived using a curve-fitting general kinetic model (GKM) algorithm. Symbols indicate patients with prolonged disease stabilisation (*), minor response (†), or partial response (‡).**

therefore expected. An increased incidence of hypertension (ranging from 2.7% to 36%, depending on dose) has been reported that was associated with the use of bevacizumab. In a meta-analysis of 1850 patients treated with bevacizumab, the incidence of proteinuria (transient albuminuria to nephrotic-range proteinuria in 1–1.8% of the cases) was reported to be from 21% to over 60% depending on the dose.[12] The underlying aetiology 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 haemolytic 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 haemolysis; 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 neovasculature,[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 VEGF[24] and can trigger an increase in mature CECs, reflecting slough-

ing 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.

## Conflict of interest statement

None declared.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.12.016.

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