Baseline Vascular Endothelial Growth Factor Concentration as a Potential Predictive Marker of Benefit from Vandetanib in Non–Small Cell Lung Cancer

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Abstract

Purpose: Vandetanib [vascular endothelial growth factor (VEGF) receptor/epidermal growth factor receptor/RET inhibitor] has shown improvements in progression-free survival (PFS) in advanced non-small cell lung cancer in three randomized phase II studies: vandetanib versus gefitinib (study 3), docetaxel ± vandetanib (study 6), and carboplatin-paclitaxel and/or vandetanib (study 7). In study 7, vandetanib monotherapy was inferior to carboplatin-paclitaxel. We performed an exploratory retrospective analysis of the relationship between baseline circulating VEGF concentrations and PFS.

Experimental Design: Mean baseline VEGF levels were determined by ELISA from two baseline samples of plasma (163 of 168 patients, study 3; 65 of 127, study 6) or serum (144 of 181, study 7). High baseline VEGF values were above the immunoassay reference range for healthy subjects; low baseline VEGF values were within the range.

Results: Patients with low baseline VEGF had a lower risk of disease progression with vandetanib versus gefitinib [hazard ratio (HR), 0.55; 95% confidence interval (95% CI), 0.35-0.86; $P = 0.01$] or vandetanib 100 mg/d + docetaxel versus docetaxel (HR, 0.25; 95% CI, 0.09-0.68; $P = 0.01$). High VEGF patients had a similar risk of disease progression with vandetanib monotherapy versus gefitinib (HR, 1.03; 95% CI, 0.60-1.75; $P = 0.92$) or vandetanib 100 mg/d + docetaxel versus docetaxel (HR, 0.95; 95% CI, 0.25-3.61; $P = 0.94$). In study 7, low VEGF patients had a similar risk of disease progression with vandetanib monotherapy 300 mg/d versus carboplatin-paclitaxel (HR, 0.80; 95% CI, 0.41-1.56; $P = 0.51$); high VEGF patients progressed more quickly (HR, 1.60; 95% CI, 0.81-3.15; $P = 0.17$).

Conclusions: These analyses suggest that low baseline circulating VEGF may be predictive of PFS advantage in patients with advanced non-small cell lung cancer receiving vandetanib versus gefitinib or vandetanib + docetaxel versus docetaxel. Moreover, patients with low VEGF levels may have a similar outcome with either vandetanib monotherapy or carboplatin-paclitaxel.

Angiogenesis is the process of new blood vessel formation from existing vessels. Generally, tumors cannot grow beyond 1 to 2 mm^3 without developing a vascular supply (1, 2). In normal physiologic processes, angiogenesis is closely controlled by the balance of proangiogenic and antiangiogenic factors, but this equilibrium is disrupted in the malignant state by the release of proangiogenic factors from the tumor and its stromal cells (3, 4). Vascular endothelial growth factor (VEGF), an up-regulated, critical proangiogenic factor in tumors, promotes endothelial cell growth, survival, and migration and mediates vessel permeability, thereby facilitating tumor progression and metastatic spread (5–7). Agents targeting the VEGF signaling pathway are

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Translational Relevance

There is a critical need for biomarkers for identifying patients likely to respond to angiogenesis inhibitors. We describe our analysis of circulating vascular endothelial growth factor (VEGF) levels from three randomized, phase II clinical studies of vandetanib (oral VEGF/epidermal growth factor receptor inhibitor) for advanced non-small cell lung cancer. Our findings have several potentially important clinical implications. Low baseline VEGF levels may identify a subset of patients who can obtain equivalent progression-free survival benefit with first-line vandetanib as with carboplatin/paclitaxel chemotherapy and could thereby be spared upfront chemotherapy. Patients with low baseline VEGF may derive greater benefit from the addition of vandetanib to second-line docetaxel chemotherapy than with docetaxel alone. Patients with low baseline VEGF who are being considered for second/third-line treatment with an epidermal growth factor receptor inhibitor may derive greater progression-free survival benefit if treated with vandetanib monotherapy. Based on these results, VEGF is being evaluated as a predictive biomarker in four phase III trials with vandetanib for non-small cell lung cancer.

now in clinical use for a variety of advanced solid tumors. These include bevacizumab, an anti-VEGF monoclonal antibody, for non-small cell lung cancer (NSCLC), colorectal and breast cancers, and sunitinib and sorafenib, multitargeted receptor tyrosine kinase inhibitors (TKI) with activity against VEGF receptors (VEGFR), for the treatment of renal cell carcinoma (8–13). Many other VEGFR TKIs are currently in clinical development (14).

Vandetanib (ZACTIMA) is a once-daily oral receptor TKI with activity against VEGFR-2, epidermal growth factor receptor (EGFR), and RET. It has shown improvements in progression-free survival (PFS) in advanced NSCLC in three randomized phase II trials (Table 1), 6474IL/0003, 0006, and 0007 (hereafter called studies 3, 6, and 7, respectively), and is now being further evaluated in the phase III setting. In study 3, there was an improvement in PFS with vandetanib 300 mg/d compared with gefitinib (IRESSA) 250 mg/d (15). Study 6 compared docetaxel alone or in combination with vandetanib at either 100 or 300 mg/d (16). PFS was superior with docetaxel + vandetanib 100 mg/d versus docetaxel alone. In study 7, combining vandetanib 300 mg/d with carboplatin-paclitaxel produced a greater PFS benefit than carboplatin-paclitaxel alone (17). In this study, the vandetanib 300 mg/d monotherapy arm was inferior to carboplatin-paclitaxel alone. Nevertheless, the disease control rate with vandetanib monotherapy was 26% (partial response or stable disease for at least 12 weeks), and a subset of patients (11%) remained on single-agent vandetanib for at least 6 months.

Whereas these phase II results show the potential of vandetanib therapy in NSCLC, the identification of pretreatment biomarkers that may predict which patients are most likely to derive the greatest benefit from vandetanib or other inhibitors of VEGF signaling is of considerable interest. Circulating VEGF levels have been shown previously to be both a prognostic marker in cancer(18) and a pharmacodynamic marker of VEGFR-2 inhibition (11, 19–21).We hypothesized that circulating VEGF levels have the potential to be a predictive marker of clinical benefit in patients with advanced NSCLC treated with vandetanib. We therefore performed exploratory analyses of pretreatment blood samples from patients enrolled in studies 3, 6, and 7 to determine if VEGF concentrations might be predictive of benefit from vandetanib monotherapy or vandetanib in combination with docetaxel or carboplatin-paclitaxel chemotherapy.

Materials and Methods

Data from three separate randomized phase II trials of vandetanib in advanced NSCLC are included in this analysis: studies 3, 6, and 7. The design and results of these trials are described in detail elsewhere and are briefly outlined here and summarized in Table 1 (15–17).

Study designs and treatments administered. In study 3, 168 patients with advanced NSCLC who had progressed despite first- or second-line platinum-based therapy were randomized 1:1 to receive continuous oral dosing with vandetanib 300 mg/d or gefitinib 250 mg/d (Table 1). The primary objective was to determine if vandetanib prolonged PFS relative to gefitinib. On disease progression, eligible patients had the option of switching to the alternative therapy.

In study 6, 127 patients with locally advanced or metastatic NSCLC who had progressed following first-line platinum-based chemotherapy were randomized 1:1:1 to one of three treatment arms: docetaxel (75 mg/m^2 intravenously every 21 days) + placebo, docetaxel + vandetanib 100 mg/d, or docetaxel + vandetanib 300 mg/d. The primary objective was to determine whether vandetanib (100 or 300 mg) + docetaxel prolonged PFS compared with placebo + docetaxel.

In study 7, 181 patients with previously untreated, locally advanced, metastatic, or recurrent NSCLC were randomized 2:1:1 to one of three treatment arms: vandetanib 300 mg/d, carboplatin-paclitaxel (carboplatin, AUC 6 mg/mL min; paclitaxel, 200 mg/m^2; intravenously every 21 days) + placebo, or carboplatin-paclitaxel + vandetanib 300 mg/d. The primary objective was to determine whether vandetanib ± carboplatin-paclitaxel prolonged PFS compared with carboplatin-paclitaxel alone.

Tumor response and disease progression were determined by Response Evaluation Criteria in Solid Tumors in all three trials, which were approved by all relevant institutional ethical committees or review bodies, and conducted in accordance with the Declaration of Helsinki, Good Clinical Practice, and the AstraZeneca policy on Bioethics. Each patient provided written informed consent.

Plasma and serum collection and preparation. Patients in studies 3 and 6 provided two baseline blood samples taken at least 24 h apart (on day 1 and usually within 7 days before commencing treatment) and which were taken into tubes containing EDTA anticoagulant. Within 30 min of collection, blood samples were centrifuged at 1,000 to 1,500 × *g* for 10 min. Plasma was frozen and stored at −70°C to −80°C. In study 7, serum was prepared from two baseline blood samples taken at least 24 h apart (on day 1 and usually within 7 days before commencing treatment). Blood samples were allowed to coagulate for 30 to 60 min and then centrifuged for 10 to 15 min at 1,000 × *g*. Serum was frozen and stored at −70°C to −80°C.

Measurement of VEGF concentration. Plasma or serum samples were thawed on ice and the VEGF concentration was determined using ELISA (R&D Systems). The VEGF standard curve ranged from 0 to 2,000 pg/mL and the lower limit of detection was 31 pg/mL. Each sample was analyzed in duplicate, and samples were analyzed in batches to minimize interassay variability.

Statistical methods. Summary statistics of baseline VEGF values were obtained for each study and each treatment group to determine the distribution of baseline VEGF values. VEGF values from two samples provided by each patient were used to obtain baseline and reproducibility measurements of the VEGF values. Where two pretreatment samples were obtained, the mean of the two VEGF values was used as the baseline measure. Where one pretreatment sample was available, this single VEGF value was used as the baseline measure. The variability between the two baseline VEGF values obtained for each patient was investigated through estimating intersubject and intrasubject components of variation using an ANOVA model fitted to the log-transformed baseline VEGF value, with patient included as a random effect.

An evaluation of different cutoff points of baseline VEGF values to predict PFS was done using a Cox proportional hazards regression model. PFS is defined as the time from randomization until progression or death in the absence of progression if death is <3 months from the last evaluable Response Evaluation Criteria in Solid Tumors assessment. Separate models were fitted for each study and different cutoff points were used to dichotomize patients into "high" and "low" VEGF subgroups. The high and low VEGF subgroups reported in this study were defined using the upper limits of VEGF concentrations reported in healthy volunteers. The VEGF concentrations in samples from 37 healthy volunteers have been reported to range from 62 to 707 pg/mL in serum and from nondetectable to 115 pg/mL in EDTA plasma (R&D Systems Human VEGF Immunoassay). Therefore, in studies 3 and 6, high and low plasma VEGF levels were defined as concentrations >115 and ≤115 pg/mL, respectively; in study 7, high and low serum VEGF levels were defined as concentrations >707 and ≤707 pg/mL, respectively.

The fitted models allowed for the effect of treatment and included terms for gender, histology, and previous response to therapy (study 3) and tumor stage and number of organs involved (studies 6 and 7). From the fitted models, hazard ratio (HR), 95% confidence interval (95% CI), and two-sided *P* value for the following five comparisons were calculated for all patients in the study and the low and high VEGF subgroups: vandetanib 300 mg versus gefitinib 250 mg (study 3), docetaxel with vandetanib 100 mg/d versus docetaxel and placebo (study 6), docetaxel with vandetanib 300 mg/d versus docetaxel and placebo (study 6), carboplatin-paclitaxel with vandetanib 300 mg/d versus carboplatin-paclitaxel and placebo (study 7), and vandetanib 300 mg/d versus carboplatin-paclitaxel and placebo (study 7). Comparisons were only between the treatment arms in each clinical study (that is, comparisons across different clinical studies were not done). A similar analysis was done using the endpoint of overall survival (OS). OS is defined as the number of days from randomization until death by any cause.

The treatment-by-VEGF interaction was investigated by assessing the difference between the log likelihoods for the full model for PFS (including all covariates, dichotomized VEGF value, and an interaction between treatment and baseline VEGF) and a reduced model for PFS (excluding the interaction). The change in $-2 \times$ log likelihood was calculated to determine whether the inclusion of the interaction term significantly improves the fit of the model; hence, the interaction is significant. *P* value(s) for the improvement of model fit are presented.

All of the phase II clinical studies were powered for the PFS primary endpoint. The VEGF analysis is therefore exploratory and multiple comparisons have been conducted, for which no adjustments have been made.

Results

Patient characteristics and baseline VEGF levels. Baseline VEGF plasma concentrations were available from the following patients: 82 of 83 (99%) in the vandetanib arm and 81 of 85 (95%) in the gefitinib arm (study 3), 24 of 41 (59%) in the

Table 1. Study designs

	Study 3	Study 6	Study 7
Study design	Two-arm, randomized phase II Second/third-line treatment for advanced NSCLC; previous platinum-based therapy	Three-arm, randomized phase II Second-line treatment for advanced NSCLC; post-failure of platinum-based therapy	Three-arm, randomized phase II First-line treatment for stage IIIB/IV NSCLC
Treatment arms	1: Gefitinib 250 mg/d, orally	1: Docetaxel 75 mg/m^2 every 21 d + placebo	1: Carboplatin AUC 6 + paclitaxel 200 mg/m^2 every 21 d (carboplatin-paclitaxel)
	2: Vandetanib 300 mg/d, orally	2: Docetaxel 75 mg/m^2 every 21 d + vandetanib 100 mg/d, orally 3: Docetaxel 75 mg/m^2 every 21 d + vandetanib 300 mg/d, orally	2: Carboplatin-paclitaxel + vandetanib 300 mg/d, orally 3: Vandetanib 300 mg/d, orally
Primary endpoint	PFS	PFS	PFS
PFS result	Primary endpoint was met	Primary endpoint was met for arm 2	Primary endpoint was met for arm 2
	Superior PFS with vandetanib	Superior PFS with docetaxel + vandetanib 100 mg compared with docetaxel + placebo	Superior PFS with carboplatin-paclitaxel + vandetanib 300 mg compared with carboplatin-paclitaxel + placebo
	HR, 0.69; *P* = 0.013 (one-sided) and 0.025 (two-sided)	HR, 0.64; *P* = 0.037 (one-sided) and 0.074 (two-sided)	HR, 0.76; *P* = 0.098 (one-sided) and 0.197 (two-sided) Arm 3 was stopped at an interim analysis (HR for PFS vs carboplatin-paclitaxel was >1.33)*
OS result	Trial had switchover design to other treatment, so OS result may be confounded	No significant difference	No significant difference

*The disease control rate with vandetanib monotherapy was 26% (partial response or stable disease for at least 12 wk) and a subset of patients (11%) remained on single-agent vandetanib for at least 6 mo.

Table 2. Summary of PFS data

	Group	PFS				
		Patients (*n*)	Events (*n*)	HR (two-sided 95% CI)	Two-sided *P*	$P_{interaction}$ (VEGF × treatment)
Study 3						
Vandetanib 300 mg/d versus gefitinib 250 mg/d	All patients	168	152	0.69 (0.50-0.96)	0.03	
	All patients with VEGF value	163	147	0.70 (0.50-0.97)	0.03	
	Low VEGF group	93	84	0.55 (0.35-0.86)	0.01	
	High VEGF group	70	63	1.03 (0.60-1.75)	0.92	0.08
Study 6						
Vandetanib 100 mg/d + docetaxel versus placebo + docetaxel	All patients	83	64	0.64 (0.39-1.05)	0.07	
	All patients with VEGF value	44	32	0.38 (0.18-0.81)	0.01	
	Low VEGF group	29	20	0.25 (0.09-0.68)	0.01	
	High VEGF group	15	12	0.95 (0.25-3.61)	0.94	0.09
Vandetanib 300 mg/d + docetaxel versus placebo + docetaxel	All patients	85	65	0.83 (0.50-1.37)	0.46	
	All patients with VEGF value	45	32	0.59 (0.29-1.21)	0.15	
	Low VEGF group	29	22	0.66 (0.28-1.54)	0.33	
	High VEGF group	16	10	0.53 (0.13-2.20)	0.38	0.71
Study 7						
Vandetanib 300 mg/d + carboplatin-paclitaxel versus placebo + carboplatin-paclitaxel	All patients	108	92	0.76 (0.50-1.15)	0.20	
	All patients with VEGF value	86	75	0.75 (0.47-1.19)	0.22	
	Low VEGF group	50	45	0.72 (0.39-1.33)	0.29	
	High VEGF group	36	30	0.47 (0.20-1.07)	0.07	0.09
Vandetanib 300 mg/d versus placebo + carboplatin-paclitaxel	All patients	113	98	1.30 (0.85-1.98)	0.23	
	All patients with VEGF value	91	79	1.27 (0.80-2.01)	0.31	
	Low VEGF group	45	37	0.80 (0.41-1.56)	0.51	
	High VEGF group	46	42	1.60 (0.81-3.15)	0.17	0.92

docetaxel arm, 20 of 42 (48%) in the docetaxel + vandetanib 100 mg/d arm, and 21 of 44 (48%) in the docetaxel + vandetanib 300 mg/d arm (study 6). Baseline serum VEGF concentrations were available for 44 of 56 (79%) patients in the carboplatin-paclitaxel + vandetanib arm and 42 of 52 (81%) in the carboplatin-paclitaxel arm (study 7). Because the vandetanib monotherapy arm in study 7 was closed at interim analysis, the subgroup of patients included in the statistical analysis of the vandetanib monotherapy arm were all patients concurrently randomized to receive either vandetanib monotherapy or carboplatin-paclitaxel up until the date when the last monotherapy patient was enrolled (August 15, 2005). For this subgroup, baseline serum VEGF concentrations were available for 58 of 73 (79%) patients in the vandetanib monotherapy arm and 33 of 40 (83%) patients in the carboplatin-paclitaxel arm. The numbers of patients in the high and low VEGF groups for each of the five comparisons between treatment arms in this analysis are shown in Table 2. The pretreatment VEGF concentrations are shown in Fig. 1 and Table 3. The intrapatient variability between the two baseline VEGF measurements taken from samples drawn on different days was low to moderate (coefficient of variation values of 36%, 55%, and 60% for studies 7, 3, and 6, respectively; Supplementary Table S1).

The patient characteristics are shown in Supplementary Table S2. There were no apparent differences between the VEGF subgroups (low or high) and the whole study population in terms of the following characteristics: treatment arm, age, performance status, and smoking status (all studies) and gender, histology, and disease stage (studies 3 and 7). In study 6, there were significant differences between the proportion of males and females in the high baseline VEGF group (33% and 67%, respectively) and the whole study group (57% and 43%, respectively) and in the proportion of patients with stage IIIB disease (14%) in the low VEGF group compared with the whole study group (26%). Study 6 also had a larger proportion of patients with adenocarcinoma (66%) and a smaller proportion of patients with squamous cell carcinoma (14%) in the low baseline VEGF group than in the whole study (50% and 29%, respectively). In addition, compared with the overall population in study 6, patients with an available baseline sample had a lower HR for benefit with vandetanib + docetaxel versus docetaxel (e.g., PFS HR of 0.64 for overall population versus 0.38 for those with baseline value in vandetanib 100 mg/d arm; Table 2; Supplementary Table S3).

Relationship between baseline VEGF levels and patient outcomes with vandetanib monotherapy. Study 3: Patients in the low baseline plasma VEGF group receiving vandetanib 300 mg/d had a superior PFS compared with those receiving gefitinib 250 mg/d (HR, 0.55; 95% CI, 0.35-0.86; two-sided P = 0.01; Figs. 2 and 3A; Table 2). In contrast, patients with high

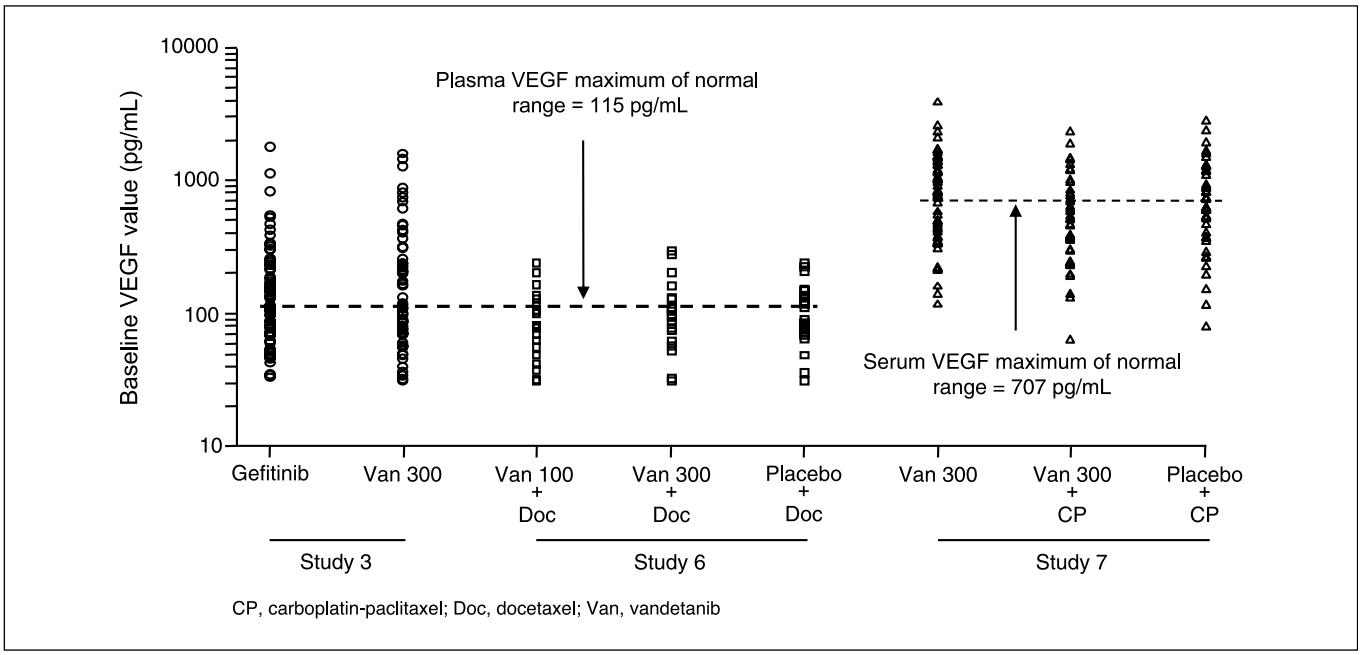

Fig. 1. Baseline VEGF values by treatment group.

baseline plasma VEGF had similar PFS when treated with either vandetanib or gefitinib (HR, 1.03; 95% CI, 0.60-1.75; $P = 0.92$; treatment-by-factor interaction test for VEGF, $P = 0.08$). Although a similar analysis was done for survival, the two-part design of the study, which allowed eligible patients the option to switch to the alternative treatment regimen in part B (following disease progression), confounds the interpretation of this analysis (Fig. 3B; Supplementary Table S3).

The finding that patients with low VEGF may derive differential PFS benefit from vandetanib compared with gefitinib was further explored to determine if the specific cutoff value for defining low or high baseline VEGF affected the overall findings. Across a broad range of cutoff points for VEGF, including the median and mean values of VEGF, the findings were similar (Fig. 4). The low baseline VEGF group consistently had a HR of <1, and the high baseline VEGF group consistently had a HR greater than that of the low VEGF group.

Study 7: The vandetanib monotherapy arm was closed at interim analysis because PFS met the criterion for discontinuation (HR > 1.33 versus carboplatin-paclitaxel); hence, the subgroup

Table 3. Baseline VEGF values by treatment group

Study	Treatment arm	Patients providing baseline samples (*n*)	VEGF (pg/mL)*					
			Arithmetic mean	Geometric mean	Maximum	Minimum†	Median	Interquartile range
3	Vandetanib 300 mg	81	180	121	1,678	31	107	138
	Gefitinib 250 mg	82	226	120	1,595	31	88	164
6	Docetaxel + placebo	24	103	86	240	31	88	79
	Docetaxel + vandetanib 300 mg	20	96	81	238	31	82	69
	Docetaxel + vandetanib 100 mg	21	108	89	296	31	86	66
7	Carboplatin-paclitaxel + placebo	42	827	630	2,781	80	674	788
	Carboplatin-paclitaxel + vandetanib 300 mg	44	664	511	2,315	64	512	500
	Vandetanib 300 mg	58	914	703	3,870	117	768	864

*VEGF levels in studies 3 and 6 are from plasma. VEGF levels in study 7 are from serum.
†The limit of VEGF quantification in the ELISA assay was 31 pg/mL. Samples at or below the limit of detection were ascribed a level of 31 pg/mL.

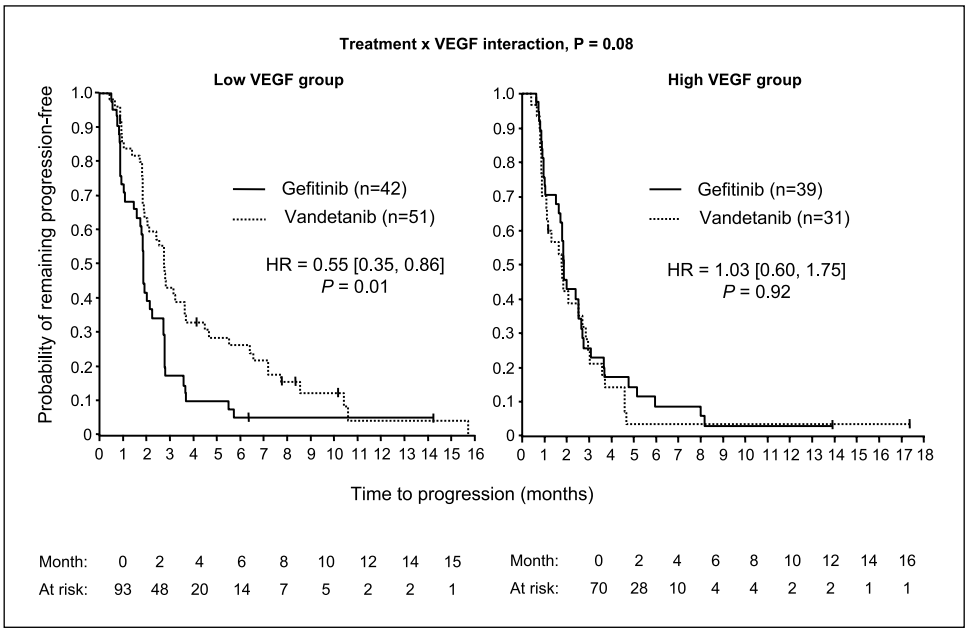

Fig. 2. Kaplan-Meier analysis for patients treated with vandetanib or gefitinib (study 3) with low or high baseline VEGF.

of patients included in this analysis were those patients concurrently randomized to receive vandetanib monotherapy (n = 73) or carboplatin-paclitaxel (n = 40) up to the date when the last monotherapy patient was enrolled. Among chemotherapy-naive NSCLC patients with low baseline serum VEGF, there was no significant difference in PFS between those initially treated with vandetanib 300 mg/d monotherapy or carboplatin-paclitaxel chemotherapy, but there was a trend toward superior PFS with vandetanib (HR, 0.80; 95% CI, 0.41-1.56; P = 0.51; Fig. 3A; Table 2). Patients with a high baseline serum VEGF tended to have an inferior PFS when treated with vandetanib 300 mg/d compared with carboplatin-paclitaxel (HR, 1.60; 95% CI, 0.81-3.15; P = 0.17; treatment-by-factor interaction test for VEGF, P = 0.09). These findings for low and high serum VEGF subgroups held across a broad range of cutoff values for VEGF, including the median and mean values of VEGF (data not shown).

Patients with a high baseline serum VEGF also trended toward having an inferior OS when treated with vandetanib 300 mg/d monotherapy compared with carboplatin-paclitaxel (HR, 1.40; 95% CI, 0.58-3.37; P = 0.46) in contrast to patients with a low baseline serum VEGF value (HR, 0.68; 95% CI, 0.29-1.60; P = 0.38; Fig. 3B; Supplementary Table S3).

Relationship between baseline VEGF levels and patient outcomes with combination therapy. Study 6: Patients in the low baseline plasma VEGF group receiving docetaxel with vandetanib 100 mg/d had a superior PFS compared with those receiving docetaxel with placebo (HR, 0.25; 95% CI, 0.09-0.68; P = 0.01; Fig. 3C; Table 2; treatment-by-factor interaction test for VEGF, P = 0.14). However, there was no significant difference in PFS among patients with low baseline plasma VEGF who received docetaxel + vandetanib 300 mg/d versus docetaxel with placebo (HR, 0.66; 95% CI, 0.28-1.54; P = 0.33). Among patients with high baseline plasma VEGF, there was no evidence of differences in PFS between the three treatment arms.

Among patients with high baseline plasma VEGF, there were also no significant differences in OS between the three treatment arms (Fig. 3D). However, there was evidence of superior OS for patients with low baseline plasma VEGF who received either vandetanib 100 mg/d (HR, 0.10; 95% CI, 0.03-0.31; P < 0.001) or vandetanib 300 mg/d (HR, 0.27; 95% CI, 0.10-0.69; P = 0.01) + docetaxel compared with docetaxel + placebo (Fig. 3D; Supplementary Table S3). A similar relationship between the dichotomous VEGF grouping and clinical outcomes of PFS and OS was observed using a median cutoff value but could not be shown across a broad range of cutoff points due to insufficient baseline VEGF data.

Study 7: Among patients with low baseline VEGF, there was no significant difference in PFS between those treated with vandetanib 300 mg/d + carboplatin-paclitaxel compared with carboplatin-paclitaxel (HR, 0.72; 95% CI, 0.39-1.33; P = 0.29; Fig. 3C; Table 2). There was a trend for patients with a high baseline serum VEGF to have superior PFS when treated with vandetanib 300 mg/d + carboplatin-paclitaxel compared with carboplatin-paclitaxel (HR, 0.47; 95% CI, 0.20-1.07; P = 0.07; treatment-by-factor interaction test for VEGF, P = 0.92). This finding was similar when the baseline VEGF median value was used as a cutoff to define the low and high VEGF groups. However, these findings were not consistent across a broad range of cutoff values for baseline VEGF. Similar results were obtained for OS between treatment arms of the high and low baseline serum VEGF groups (Fig. 3D; Supplementary Table S3).

Discussion

In this exploratory retrospective analysis of pretreatment plasma or serum VEGF concentrations among the participants in three randomized phase II studies, we have evaluated baseline circulating VEGF level as a potential predictive marker for clinical benefit from vandetanib treatment either as a monotherapy or in combination with chemotherapy for advanced NSCLC. From the study 3 results, we have shown that patients with advanced NSCLC and a low baseline plasma VEGF concentration had a significantly superior PFS when treated with vandetanib monotherapy compared with gefitinib monotherapy

(HR, 0.55; $P = 0.01$). In contrast, patients with a high baseline concentration of VEGF had a similar risk of tumor progression when treated with either vandetanib or gefitinib (HR, 1.03; $P = 0.92$). Due to the two-part crossover design of this study, it is not possible to make any definitive conclusions about the effect of therapy on OS. In addition, NSCLC patients with low baseline serum VEGF treated in the first-line setting in study 7 appeared to derive similar benefit in terms of both PFS and OS from either vandetanib monotherapy or carboplatin-paclitaxel doublet chemotherapy, an established standard of care, whereas patients with high VEGF had a shorter PFS with vandetanib. This suggests that determining pretreatment circulating VEGF concentrations may have the potential to identify patients who could derive equivalent benefit from front-line targeted therapy with vandetanib monotherapy as from standard carboplatin-paclitaxel doublet chemotherapy.

Baseline VEGF levels may also have the potential to identify patients most likely to benefit from the addition of vandetanib to chemotherapy, particularly for vandetanib 100 mg/d + docetaxel compared with docetaxel alone in the second-line treatment of NSCLC. In this setting, patients with low baseline plasma VEGF treated with vandetanib 100 mg/d + docetaxel

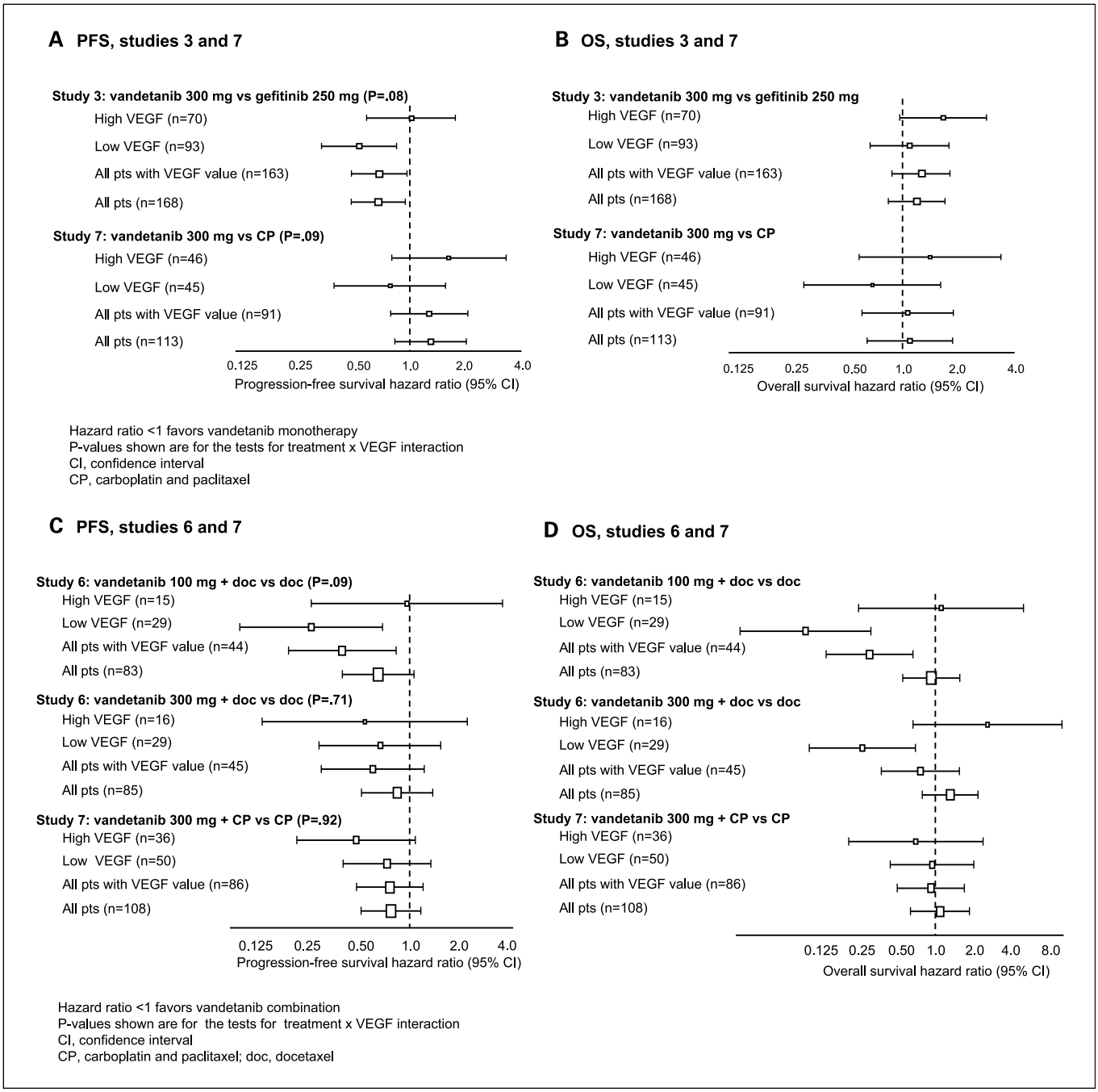

Fig. 3. PFS and OS for patients with low or high baseline VEGF treated with vandetanib monotherapy (studies 3 and 7) or vandetanib combination therapy (studies 6 and 7).

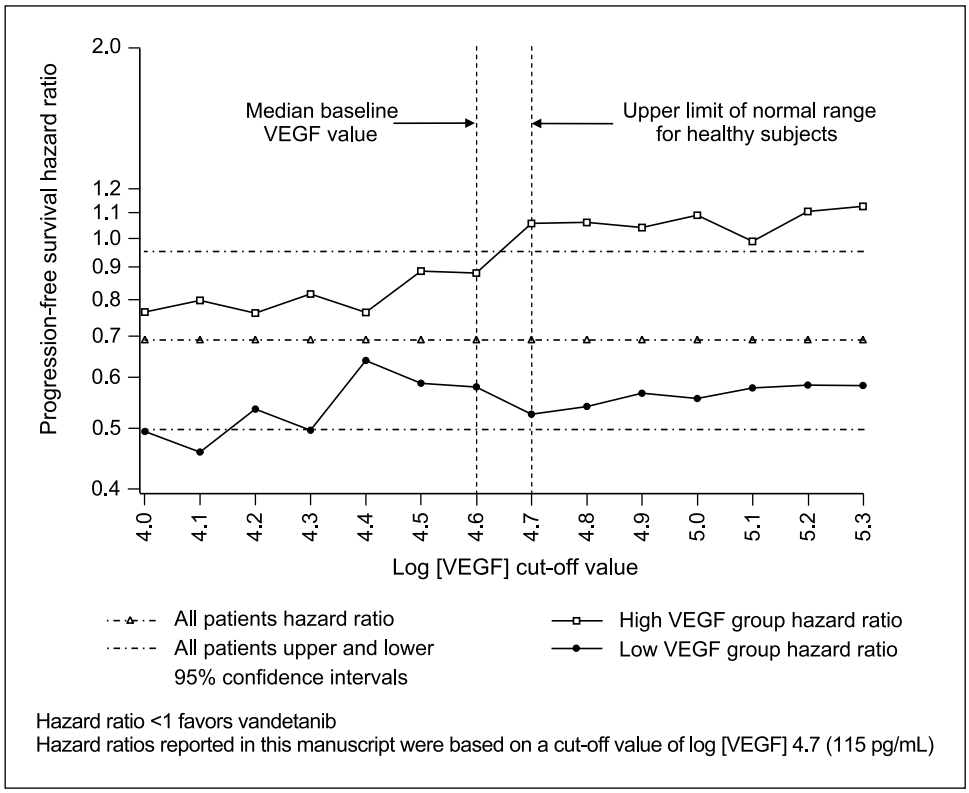

Fig. 4. Exploration of different cutoff points to dichotomize patients into low or high baseline VEGF groups for study 3 (PFS).

appeared to have superior PFS and OS outcomes compared with those treated with docetaxel alone, whereas patients with high baseline VEGF had similar treatment outcomes in both arms. The results were more complex for vandetanib 300 mg/d in combination with chemotherapy. For vandetanib 300 mg/d in combination with docetaxel versus docetaxel alone, there was a superior OS, but not PFS, outcome for previously treated NSCLC patients with low baseline VEGF. For vandetanib 300 mg/d in combination with carboplatin-paclitaxel versus carboplatin-paclitaxel alone, there was some evidence that previously untreated patients with high levels of pretreatment serum VEGF may have gained greater benefit in terms of both PFS and OS. One possible explanation for these observations is that the predictive value of VEGF may depend, at least in part, on the vandetanib dose. This reflects the observed clinical trial results with vandetanib in combination with chemotherapy. In studies 6 and 7, the addition of vandetanib 300 mg/d to docetaxel and carboplatin-paclitaxel, respectively, yielded modest PFS benefits compared with chemotherapy alone (HR, 0.83 and 0.76, respectively), and the greatest PFS benefit was seen when vandetanib 100 mg/d was added to docetaxel (HR, 0.64; refs. 16, 17). As shown in four randomized phase III studies, the addition of EGFR TKIs to chemotherapy for NSCLC does not improve outcome (22–25). It is thought that EGFR TKIs induce G_1 cell cycle arrest and thereby reduce the efficacy of cell cycle-dependent cytotoxic agents (26). It has been theorized that the EGFR inhibitory capacity of vandetanib predominates at higher doses, thereby limiting the benefit of adding this agent at the 300 mg/d dose to chemotherapy (16). At the lower 100 mg/d dose, there may be less EGFR inhibitory effect, such that the anti-VEGFR effects of vandetanib and the cytotoxic effects of chemotherapy can predominate (16). This theory is in keeping with our findings that VEGF levels may only be associated with outcome when the lower dose of vandetanib is combined with chemotherapy.

We defined "high" and "low" VEGF levels as being either above or below, respectively, the upper limit of normal reported in analyses of blood by ELISA from healthy subjects (R&D Systems Human VEGF Immunoassay). Although our findings were similar across a range of cutoff values for high versus low VEGF, including the median VEGF concentration in all three studies, we chose to present the data using this predefined, fixed cutoff point rather than the median VEGF level. Our rationale for this was that a fixed cutoff point for VEGF level could be more easily applied to clinical practice than the median concentration, which would be expected to vary between different groups of patients, if circulating VEGF level is validated as a predictive biomarker and becomes incorporated into future clinical decision-making processes.

Increased circulating VEGF concentration in patients with NSCLC and other solid tumors has been shown to correlate with poor prognosis (18, 27), and although VEGF levels in blood correlated with VEGF expression in tumor tissue from patients with colorectal cancer (28), this was not the case in NSCLC or breast cancer (29, 30). The origin of circulating VEGF measured by ELISA in plasma (studies 3 and 6) or serum (study 7) in the present analyses has not been determined but may include contributions from both tumor and nontumor tissues (31). In addition, platelet disruption, particularly during the preparation of serum, is likely to contribute to the overall levels of circulating VEGF measured in the present study (32), although whether platelet-derived VEGF originates from tumor or other tissues remains to be determined (33). However, irrespective of the origin of plasma or serum VEGF, it was unanticipated that our analyses showed that low,

rather than high, circulating VEGF levels before treatment appeared to be predictive of clinical benefit from vandetanib monotherapy. One potential explanation is that, in patients with high VEGF levels, vandetanib is unable to achieve an adequate degree of VEGFR inhibition and blockade of tumor angiogenesis; alternatively, high VEGF may be associated with high levels of other angiogenic factors, such as basic fibroblast growth factor and interleukin-8, which are able to sustain angiogenesis even in the presence of VEGFR blockade. Consistent with our findings, baseline plasma VEGF levels were recently reported to be lower in Japanese NSCLC patients who experienced clinical benefit from vandetanib than those who did not in a phase II trial (34). However, because all patients received vandetanib, it was not possible to determine whether baseline plasma VEGF was a prognostic or predictive biomarker. However, because all patients received vandetanib, it was not possible to determine whether baseline plasma VEGF was a prognostic or predictive biomarker in this setting.

A blood-based predictive biomarker has many practical and safety advantages over tissue- or imaging-based predictive biomarkers. Although there is considerable debate about whether serum or plasma is the better medium in which to assess factors such as VEGF (18), both plasma VEGF concentrations from studies 3 and 6 and serum VEGF concentrations from study 7, as determined by ELISA, were predictive in our analyses. We did not have both plasma and serum suitable for analysis by ELISA from the participants in these randomized trials of vandetanib, so we cannot draw any conclusions about the relative benefits of each of these types of blood sample in assessing circulating VEGF.

With regards to future potential clinical application of blood-based biomarkers, it may be most practical to identify a single factor that can predict benefit from VEGFR TKIs, such as VEGF, but in reality it is likely that baseline levels of other circulating angiogenic factors or cytokines also have predictive values in identifying patients who will benefit most from these therapies. Therefore, a limitation of the current work is that VEGF was the only angiogenic biomarker measured across all three clinical studies. For example, low baseline plasma intercellular adhesion molecule-1 predicted a greater PFS benefit from the addition of bevacizumab to carboplatin-paclitaxel compared with carboplatin-paclitaxel alone in the randomized phase III Eastern Cooperative Oncology Group 4599 trial (35). In squamous cell carcinoma of the head and neck treated with chemoradiation, baseline levels and coordinate changes in multiple cytokines, including VEGF, were associated with poor outcomes (36). This suggests that VEGF may be one of several circulating factors that alone, or in combination with other cytokines or angiogenic factors, may be prognostic or predictive of clinical benefit following treatment with vandetanib or other VEGFR signaling inhibitors. Our preliminary data using multiplex beads to assess >30 factors from the plasma of patients in trial 7 is consistent with this possibility, as several other factors were found to potentially be predictive of vandetanib benefit (37).

Several other phase II and III studies of VEGF signaling inhibitors in a variety of solid tumor types have considered the predictive value of pretreatment circulating VEGF levels, although the findings have been somewhat inconsistent (18, 35, 38–41). The addition of bevacizumab, a monoclonal antibody targeting VEGF, to bolus 5-fluorouracil and irinotecan as first-line treatment for metastatic colorectal cancer significantly improved PFS and OS, but survival benefit was unrelated to pretreatment plasma VEGF concentration (38). Similarly, in the Eastern Cooperative Oncology Group 4599 trial of carboplatin-paclitaxel with or without bevacizumab in NSCLC, high baseline plasma VEGF was associated with a greater response rate with the use of bevacizumab but not with improved survival (35). Patients with renal cell carcinoma treated second-line with sorafenib showed a significant improvement in PFS relative to placebo, but baseline plasma VEGF was not predictive of PFS benefit from sorafenib (39). In a randomized phase II trial, the addition of bevacizumab to first-line chemotherapy with gemcitabine and cisplatin for inoperable malignant mesothelioma did not improve outcome, but on subset analyses, patients with low pretreatment VEGF plasma concentrations derived PFS and OS benefits from the use of bevacizumab, whereas patients with high plasma VEGF levels did not benefit from bevacizumab (40). These inconsistent reports regarding the predictive value of VEGF may in part arise due to the small patient numbers in such retrospective analyses. It is also conceivable that baseline VEGF levels are not a general predictive factor for benefit from VEGF signaling inhibitors, but rather baseline VEGF may be predictive in only certain tumor types and/or with only some agents. For example, if high VEGF levels counterbalance the effects of vandetanib, VEGF levels may not be predictive of benefit from a more potent VEGFR TKI.

In our analysis, the association between low baseline VEGF and benefit from vandetanib, either as monotherapy or at the 100 mg/d dose combined with chemotherapy, was generally consistent across all three clinical trials. However, no definitive conclusions about the role of VEGF as a predictive biomarker for benefit from vandetanib can be drawn from these data. The *P* values for the interaction tests did not reach statistical significance, although they trended toward significance where associations between low VEGF and treatment outcome were found (study 3, $P = 0.08$; study 7, vandetanib versus carboplatin-paclitaxel, $P = 0.09$; study 6, vandetanib 100 mg/d, $P = 0.14$). These analyses of VEGF are exploratory; therefore, the clinical studies were not powered for these interaction tests. The numbers of patients in studies 3, 6, and 7 are modest, and there is the potential for case selection bias in study 6 because VEGF data were available for only half of the study's participants. Nevertheless, our data suggest that baseline circulating VEGF may be a potential predictive biomarker for benefit from vandetanib and that further study of VEGF in large phase III trials is warranted. If our findings are validated, they could have several potentially important clinical implications. Firstly, low baseline circulating VEGF levels may identify a subset of patients with advanced NSCLC who can receive oral vandetanib as a first-line treatment rather than intravenous administration of carboplatin-paclitaxel doublet chemotherapy. In the second-line setting, advanced NSCLC patients with low baseline VEGF levels may derive greater PFS benefit from the addition of vandetanib 100 mg/d to docetaxel chemotherapy rather than with docetaxel alone. Furthermore, patients with a low baseline VEGF levels who are being considered for treatment with a selective EGFR inhibitor in the second/third-line setting may derive greater PFS benefit if treated with vandetanib monotherapy.

Based on the findings in this exploratory analysis, baseline VEGF will be evaluated as a potential predictive biomarker of clinical benefit with vandetanib in four ongoing phase III trials

in advanced NSCLC: (*a*) second/third-line erlotinib versus vandetanib 300 mg/d, (*b*) second-line docetaxel ± vandetanib 100 mg/d, (*c*) second-line pemetrexed ± vandetanib 100 mg/d, and (*d*) second/third-line placebo versus vandetanib in patients who have progressed on prior treatment with an EGFR TKI (42).

Disclosure of Potential Conflicts of Interest

A.J. Ryan, H. Mann, S.J. Kennedy, P. Langmuir, employment, AstraZeneca. J.V. Heymach, R.B. Natale, R.S. Herbst, commercial research funding, consultant, AstraZeneca. B.E. Johnson, consultant, Genzyme. R.S. Herbst, honoraria, AstraZeneca.

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Baseline Vascular Endothelial Growth Factor Concentration as a Potential Predictive Marker of Benefit from Vandetanib in Non–Small Cell Lung Cancer

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