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Clinical Cancer Research

Predictive Biomarkers and Personalized Medicine

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Predictive Impact of Circulating Vascular Endothelial Growth Factor in Four Phase III Trials Evaluating Bevacizumab

Priti S. Hegde[1], Adrian M. Jubb[2], Dafeng Chen[3], Nicole F. Li[3], Y. Gloria Meng[4], Coen Bernaards[3], Rebecca Elliott[5], Stefan J. Scherer[7], and Daniel S. Chen[6]

Abstract

Purpose: We evaluated the prognostic and predictive use of circulating VEGF-A levels in phase III trials of bevacizumab in colorectal cancer, lung cancer, and renal cell carcinoma.

Methods: Baseline plasma samples from 1,816 patients were analyzed for VEGF-A using an ELISA, which recognizes the major isoforms with equivalent sensitivity. HR and 95% confidence intervals (CI) for study end points were estimated using Cox regression analysis. A subset of matched archival tumor samples was analyzed for VEGF-A expression using *in situ* hybridization.

Results: Higher VEGF-A levels showed trends toward adverse prognostic significance in the control arms of multiple trials, reaching statistical significance for overall survival (OS) in AVF2107 (highest vs. lowest 50%: HR = 1.76; 95% CI, 1.28–2.41), AVAiL (HR = 1.52; 95% CI, 1.16–2.00), and AVOREN (HR = 1.67; 95% CI, 1.18–2.36). In predictive analyses, the HRs for progression-free survival were similar across low and high VEGF-A subgroups and favored bevacizumab-containing treatment. In the low VEGF-A subgroups, HRs (95% CIs) were 0.61 (0.43–0.87) in AVF2107, 0.71 (0.43–1.16) in E4599, 0.74 (0.59–0.94) in AVAiL (low-dose), 0.89 (0.70–1.13) in AVAiL (high-dose), and 0.56 (0.40–0.78) in AVOREN. Analyses of OS data have shown similar results. No correlation between primary tumor VEGF-A expression and plasma VEGF-A levels was observed.

Conclusions: In this comprehensive evaluation, pretreatment total circulating VEGF-A was prognostic for outcome in metastatic colorectal, lung, and renal cell cancers, but it was not predictive for bevacizumab-based treatment benefit. *Clin Cancer Res; 19(4); 929–37. ©2012 AACR.*

Introduction

VEGF-A, which exists in humans in multiple isoforms (1), is a proangiogenic ligand that is upregulated in a large proportion of primary malignancies (2). Tumor expression levels of VEGF-A have been correlated with vascularization, pathologic stage, metastasis, and poor outcome in patients with metastatic colorectal cancer (mCRC), non–small cell lung cancer (NSCLC), and metastatic renal cell carcinoma (mRCC; refs. 3–10). In addition, circulating VEGF-A levels are elevated in a proportion of patients with carcinomas, and some reports have suggested an association between circulating VEGF-A levels and patient outcomes (11).

The monoclonal antibody bevacizumab, which selectively inhibits VEGF-A signaling, has been extensively examined across multiple tumor types in both combination and single-agent trials. Phase III studies have shown that the addition of bevacizumab to standard chemotherapy regimens significantly improves progression-free survival (PFS) and overall survival (OS) in patients with mCRC and advanced nonsquamous NSCLC (12–14). Treatment containing bevacizumab in previously untreated mRCC has also been associated with significant improvements in PFS compared with immunotherapy alone (15, 16). Positive data from clinical trials of multitargeted agents that also inhibit VEGF-A signaling, such as the tyrosine kinase inhibitors sunitinib (17) and sorafenib (18), further underscore the value of antiangiogenic strategies in cancer treatment.

Nevertheless, no known association exists between the survival benefit and the response rate to bevacizumab-containing therapy in mCRC (19, 20), showing the need for biomarkers to better identify those who will derive the greatest incremental benefit. Biomarker analyses suggest that bevacizumab-containing therapy confers clinical benefit irrespective of the status of k-ras, b-raf, p53 (21–23), and thrombospondin-2 (24). Clinical outcome with targeted agents may be influenced by the expression level of the

Authors' Affiliations: Departments of [1]Oncology Biomarkers, [2]Pathology, [3]Biostatistics, [4]Biochemical and Cellular Pharmacology, [5]BioAnalytical Sciences, and [6]Oncology Early Clinical Development, Genentech, Inc., South San Francisco, California; and [7]F. Hoffman-La Roche AG, Basel, Switzerland

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Adherence to REMARK criteria: The authors confirm that this retrospective analysis conforms to REMARK criteria.

Corresponding Author: Priti S. Hegde, Oncology Biomarkers, MS 461a, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080. Phone: 650-225-6145; Fax: 650-742-5132; E-mail: hegde.priti@gene.com

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Translational Relevance

There has been considerable debate over whether circulating VEGF-A is a predictive marker for the use of bevacizumab-containing treatment. This is the first comprehensive analysis of plasma VEGF-A as a biomarker across multiple phase III trials. We analyzed samples from 1,816 patients with mCRC, NSCLC, or mRCC in phase III trials of standard chemotherapy or immunotherapy with or without bevacizumab to determine the prognostic and predictive use of plasma VEGF-A. High VEGF-A levels showed modest prognostic significance for survival outcomes in the overall and placebo-treated patient populations. The benefit of treatment with bevacizumab-containing regimens, however, was observed regardless of the VEGF-A level. No correlation was found between circulating and tumor VEGF-A levels. Caveats to these analyses include the tumor types evaluated and the specificity of the assay used. Biomarker identification can further improve the benefit-to-risk profile and the cost-effectiveness of molecular-targeted therapy.

target (e.g., trastuzumab and HER2; ref. 25). Therefore, VEGF-A is a strong candidate for predicting the survival benefit associated with bevacizumab treatment. Tumor VEGF-A levels, however, have not been found to predict the survival benefit of bevacizumab in retrospective subset analyses (24, 26–28).

Plasma markers offer a number of advantages over tissue-based markers, including the ability to carry out continuous, noninvasive assessments over time, which may be more relevant to the metastatic tumor being treated than to the tissue-based measurements of VEGF-A in archival primary tumors. Earlier research failed to show a difference in the survival benefit afforded by bevacizumab in subsets of patients with different circulating levels of VEGF-A (11, 29). Unfortunately, interpretation of these initial studies is limited as they describe small cohorts or single clinical trials that lack statistical power in retrospective subset analyses.

To assess the role of VEGF-A in predicting benefit from bevacizumab in as rigorous a manner as possible, we used a standardized approach to analyze plasma VEGF-A levels from patients in 5 randomized trials, including 4 phase III trials, of bevacizumab in mCRC, NSCLC, and mRCC. While evaluating the possible prognostic and/or predictive use of circulating VEGF-A levels in these studies, we also examined the relationship between plasma and tumor VEGF-A levels. This is the first report of a multistudy analysis of plasma VEGF-A as a biomarker of response to bevacizumab treatment.

Patients and Methods

Study design

These retrospective analyses meet the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria. The sample size was chosen to maximize statistical power in an exploratory fashion, not to detect a prespecified effect size. Therefore, this study included all available randomized clinical trials of bevacizumab with pretreatment plasma samples. The AVF2107 (NCT00109070), E4599 (NCT00021060), AVAiL (BO17704; NCT00806923), AVOREN (NCT00738530), and AVF2938 (NCT00081614) studies were conducted in accordance with local laws, the Declaration of Helsinki, and the U.S. Food and Drug Administration Good Clinical Practices. The study protocols were reviewed and approved by the Institutional Review Boards of all recruiting centers, and all patients provided informed consent. Collection of baseline plasma samples for measurement of VEGF-A level was prespecified in each study protocol. Detailed descriptions of the design and patient populations of these 5 studies have been published previously and are shown briefly in Table 1.

Sample collection

In all studies, patient consent was obtained before sample collection, and plasma was collected from patients at baseline. Plasma was also collected from 40 healthy donors who consented to exploratory analysis. After collection, 2 to 3 mL of citrated plasma was stored at −80°C. These samples were shipped between sites on dry ice. Upon aliquoting, several hundred microliters (depending on the recipient) were transferred into 96-well plates or microtube racks. These aliquots were stored at −80°C until distribution to different sites on dry ice. Formalin-fixed paraffin-embedded archival tissue samples and matched baseline plasma samples were obtained from patients enrolled in the AVF2107 (12, 21) and AVF2938 (30) studies.

Assays

All experiments and analyses were carried out at Genentech where laboratory scientists were blinded to treatment group and clinical outcome. The plasma VEGF-A ELISA assay GEN.038 was designed to recognize all major isoforms of VEGF-A, including $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{110}$ (the plasmin-cleaved product of $VEGF_{165}$) with equivalent sensitivity (Supplementary Figs. S1 and S2). The assay uses the murine anti–VEGF-A antibody A4.6.1 (the mouse antibody from which bevacizumab derives) to capture and detect VEGF-A. ELISAs were conducted in 96-well plates (Nalge Nunc International) coated with mouse monoclonal antibodies 5C3 and A4.6.1 that were stored overnight at between 2°C and 8°C. The plates were blocked with 0.5% bovine serum albumin (BSA), 0.05% polysorbate 20, and 0.05% ProClin 300 (Rohm and Haas) in PBS for 1 to 3 hours at room temperature. $VEGF_{165}$ calibrators, controls, and citrated plasma samples diluted 1:5 and 1:10 in sample diluent (PBS, 0.5% BSA, 0.05% polysorbate 20, 0.05% ProClin 300, 5 mmol/L ethylenediaminetetraacetic acid, 0.35 mol/L sodium chloride, 0.5 mg/mL murine immunoglobulin G) were added to the plate and incubated for 1.5 to 2 hours at 37°C. The plates were washed with

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Table 1. Randomized studies of bevacizumab-based treatment for prognostic and predictive analyses

Trial	Patient Population	Regimen	Primary End Point	Result for Primary End Point	Plasma Samples
AVF2107[12]	Previously untreated mCRC ($n = 813$)	IFL with bevacizumab (5 mg/kg q2w) or placebo	OS	20.3 vs. 15.6 months (HR, 0.66; $P < 0.001$)	$n = 384$
E4599[14]	Newly diagnosed stage IIIb (malignant plural effusion) or stage IV or recurrent nonsquamous NSCLC ($N = 878$)	Carboplatin and paclitaxel with bevacizumab (15 mg/kg q3w) or placebo	OS	12.3 vs. 10.3 months (HR, 0.79; $P = 0.003$)	$n = 166$
AVAiL[36]	Stage IIIb (supraclavicular lymph node metastasis or malignant pleural effusion or pericardial effusion) or stage IV or recurrent nonsquamous NSCLC ($N = 1,043$)	Cisplatin and gemcitabine with low-dose bevacizumab (7.5 mg/kg q3w), high-dose bevacizumab (15 mg/kg q3w), or placebo	Unstratified PFS	Low-dose: 6.7 vs. 6.1 months (HR, 0.75; $P = 0.003$); High-dose: 6.5 vs. 6.1 months (HR, 0.82; $P = 0.03$)	$n = 882$
AVOREN[15]	Previously untreated, predominantly clear cell mRCC ($N = 649$)	Interferon alfa-2a with bevacizumab (10 mg/kg q2w) or placebo	PFS[a]	10.2 vs. 5.4 months (HR, 0.63, $P = 0.0001$)	$n = 384$
AVF2938[30]	Previously untreated mRCC of predominantly clear cell histology with prior nephrectomy ($N = 104$)	Bevacizumab (10 mg/kg q2w) with erlotinib or placebo	PFS	9.9 vs. 8.5 months (HR, 0.86; $P = 0.58$)	$n = 103$

Abbreviations: IFL, irinotecan with bolus fluorouracil and leucovorin; q2w, every 2 weeks, q3w, every 3 weeks.
[a]The primary end point was OS; however, the preplanned final analysis of PFS was deemed acceptable for regulatory submission.

buffer containing PBS, 0.05% polysorbate 20. Bound VEGF-A was detected with biotinylated A4.6.1, followed by streptavidin-conjugated β-galactosidase (Merck KGaA). Fluorescence was read at 360 nm for excitation absorbance and 450 nm for emission. The lower and upper limits of quantitation in the assay were 2.5 and 88.9 pg/mL, respectively. The quantifiable range in a sample is 12.5 to 889 pg/mL. The percent recovery of $VEGF_{165}$ added to citrated plasma from 6 patients with mCRC ranged from 80% to 108%. Interassay coefficients of variability (CV) ranged from 17% to 21%, and intraassay CV ranged from 7% to 16%. The A4.6.1 antibody used in the GEN.038 plasma VEGF-A ELISA shares the same epitope as bevacizumab; thus, assay interference from VEGF-A receptors fms-related tyrosine kinase 1 (FLT-1), kinase insert domain receptor (KDR), and soluble neuropilin 1 (sNRP1) was also evaluated. While sNRP1 does not interfere with assay performance, interference was observed for FLT-1 and KDR at levels of 125 pg/mL of $VEGF_{165}$, $VEGF_{121}$, and $VEGF_{110}$ and at a molar ratio of VEGF-A to FLT-1 of approximately 1:1 and at a molar ratio of VEGF-A to KDR of approximately 1:10.

Tissue microarrays to measure VEGF-A mRNA expression by *in situ* hybridization (ISH) were assembled as described previously (31). For tissue microarrays measuring VEGF-A mRNA expression, riboprobe synthesis, hybridization,

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development, and analysis were carried out as described previously (24, 32). Hybridization of antisense β-actin riboprobes was confirmed in all tissues. Sense riboprobes were used as negative controls for hybridization specificity. Tissue microarray cores were scored semiquantitatively on a scale of 0 (no expression) to 3 (very strong signal), according to the overall intensity of the hybridization signal in 10% or more of neoplastic cells. The highest score among replicate tissue microarray cores was chosen as the score for the patient. Microarray data for tumor and matched normal breast, lung, colon, and kidney tissue VEGF-A mRNA expression were obtained from GeneLogic. Probeset 210512_s_at was chosen to represent VEGF-A expression.

Statistical analyses

Patients with a baseline plasma sample and a valid total plasma VEGF-A result were included in the biomarker analysis population. Patients with missing data were excluded from analyses. Patient baseline characteristics were summarized in the biomarker population and compared with all patients enrolled in each study. The interaction between baseline plasma VEGF-A levels and PFS or OS was analyzed to determine whether it was a prognostic or predictive relationship: PFS was defined as the time from randomization until disease progression or death from any cause, whereas OS was defined as the time from randomization until death from any cause.

Continuous data were categorized into high- versus low-plasma VEGF-A levels by using a median cut point. To identify the prognostic value of baseline VEGF-A level, a stratified log-rank test was used to assess differences in the distributions of PFS and OS in the lower and upper median in placebo-treated patients. A *P*-value of less than 0.05 was considered to be statistically significant. Median PFS and OS values, together with 95% confidence intervals (CI), for patients with pretreatment VEGF-A levels were estimated according to median VEGF-A level using the Kaplan–Meier method. In the predictive analysis, median PFS and OS values were estimated using the Kaplan–Meier method; hazard ratios (HR) and 95% CI for PFS and OS for bevacizumab- and placebo-treated patients in the low-plasma and high-plasma VEGF-A groups were produced by a multivariable Cox regression model adjusted for baseline stratification factors that were used for randomization.

Results

Patient demographics and sampling

Plasma samples from 384 patients (42%) in AVF2107, 166 patients (19%) in E4599, 882 patients (85%) in AVAiL, and 384 patients (59%) in AVOREN were available for analysis. Ninety-seven matched tumor samples from AVF2107 and 35 from AVF2938 were also available for ISH. In each of the randomized phase III studies, the demographic, clinical, and pathologic characteristics of sampled patient groups were similar to those found in the population with and without available VEGF-A samples (Supplementary Table S1).

Plasma and tissue VEGF-A levels

Plasma samples from patients in the 4 phase III studies were analyzed for total circulating VEGF-A levels. The observed distribution of patients by circulating VEGF-A level was similar across all 3 tumor types (Fig. 1), with at least 34% of patients in the mCRC, NSCLC, and mRCC studies having a VEGF-A level of 50 pg/mL or more. Median circulating VEGF-A concentrations were 44 pg/mL, 36 pg/mL, 45 pg/mL, and 55 pg/mL in AVF2107, E4599, AVAiL, and AVOREN, respectively. In contrast, median circulating VEGF-A concentration in 40 healthy subjects was below the limit of quantitation (12.5 pg/mL). The VEGF concentrations in the 17 subjects with detectable VEGF levels did not exceed 49 pg/mL.

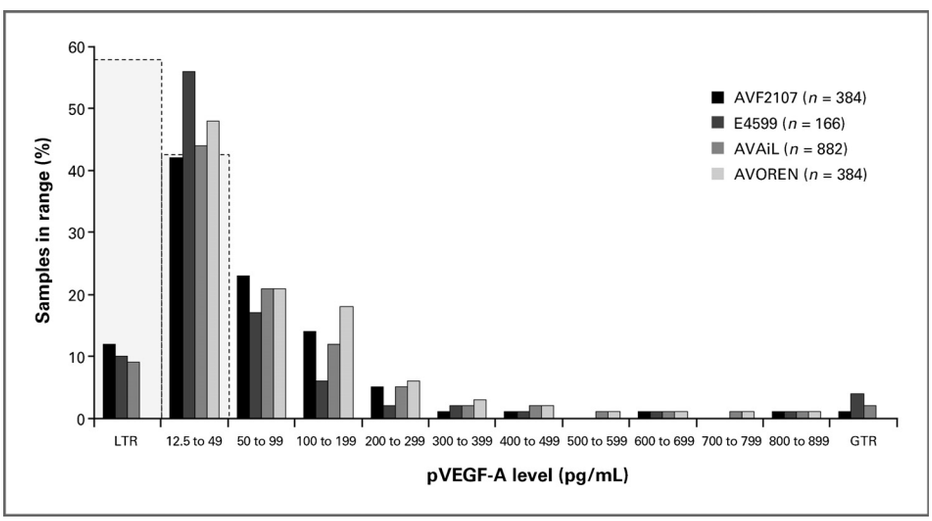

Figure 1. Distribution by baseline pVEGF-A level in the AVF2107, E4599, AVAiL, and AVOREN trials. Distribution among healthy volunteers ($n = 40$) is depicted by the dashed boxes. LTR, lower than resolution; GTR, greater than resolution; pVEGF-A, plasma vascular endothelial growth factor.

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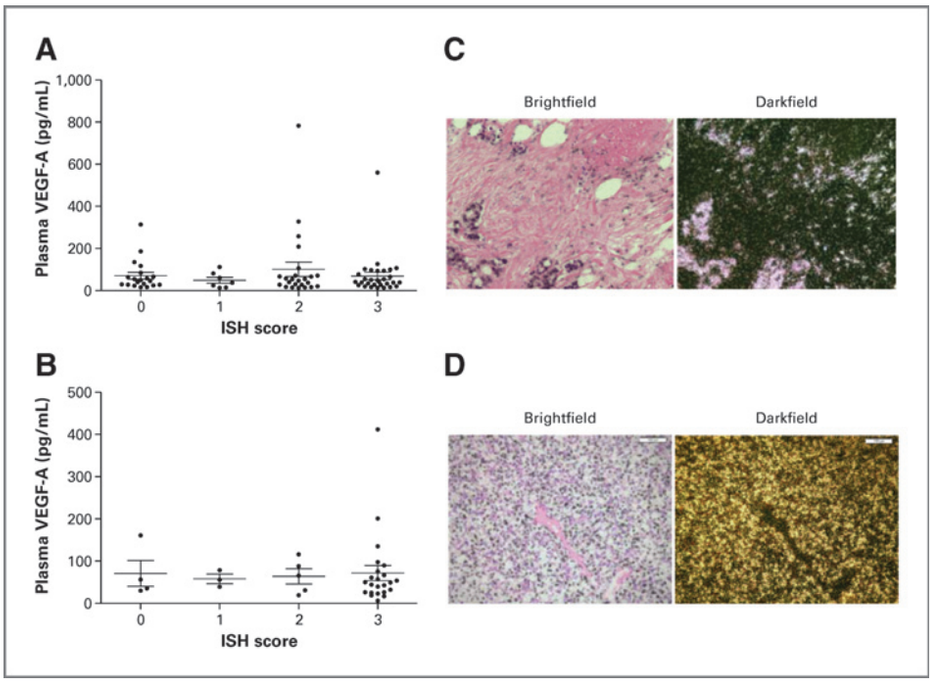

Figure 2. Plasma and tumor VEGF-A levels in AVF2107 ($n = 97$) and AVF2938 ($n = 35$) as determined by ELISA and ISH, respectively. A, in AVF2107, median values were 30, 37, 47, and 36 pg/mL in the 0, 1, 2, and 3 ISH groupings, respectively. B, in AVF2938, median values were 46, 56, 66, and 50 pg/mL (range, 6–412 pg/mL) in the 0, 1, 2, and 3 ISH groupings, respectively. C, representative brightfield and darkfield images from AVF2107 (mCRC) show sliver grain deposition over cells expressing VEGF-A mRNA against a hematoxylin and eosin counterstain, with a score of 3. D, representative brightfield and darkfield images from AVF2938 (mRCC) show sliver grain deposition over cells expressing VEGF-A mRNA against a hematoxylin and eosin counterstain, with a score of 3. Bar, 100 μmol/L. ELISA, enzyme-linked immunosorbent assays; ISH, *in situ* hybridization; mCRC, metastatic colorectal carcinoma; mRCC, metastatic renal cell carcinoma; VEGF-A, vascular endothelial growth factor.

There was no evidence of an association between circulating and tumor VEGF-A levels according to matched plasma and tumor samples from AVF2107 and AVF2938 (Fig. 2). A detailed overview of this assay and its scoring was published elsewhere (24).

Association of circulating VEGF-A levels with patient outcome

To assess the prognostic significance of circulating VEGF-A levels, low and high circulating VEGF-A subsets by median were analyzed for placebo-treated patients with available

Table 2. PFS and OS by median of circulating VEGF-A for patients in the control group of the AVF2107, E4599, AVAiL, and AVOREN trials

		PFS			OS		
Study	***n***	**Median, mo**	**HR (95% CI)**	***P***	**Median, mo**	**HR**	***P***
AVF2107 (mCRC)							
Lowest 50%	101	6.93	Reference	0.1481	17.97	Reference	
Highest 50%	90	5.52	1.28 (0.92–1.80)		12.88	1.76 (1.28–2.41)	0.0005
E4599 (NSCLC)							
Lowest 50%	37	5.59	Reference	0.4904	10.48	Reference	
Highest 50%	42	3.91	1.19 (0.72–1.96)		8.54	1.31 (0.83–2.09)	0.2510
AVAiL (NSCLC)							
Lowest 50%	143	6.44	Reference	0.1964	15.80	Reference	
Highest 50%	153	5.95	1.17 (0.92–1.47)		10.55	1.52 (1.16–2.00)	0.0026
AVOREN (RCC)							
Lowest 50%	95	7.43	Reference	0.2684	28.42	Reference	
Highest 50%	95	3.81	1.20 (0.87–1.65)		15.28	1.67 (1.18–2.36)	0.0035

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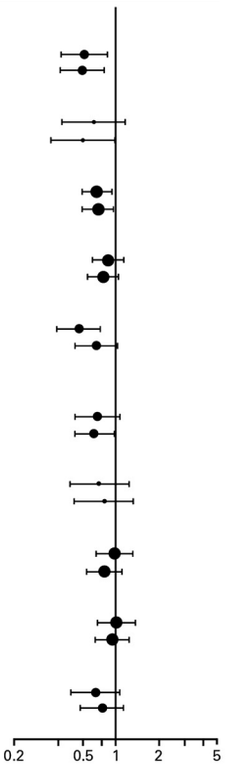
Study, Measure	Chemo[a]+PL Median (months)	Chemo+BV Median (months)	HR (95% CI)
PFS			
AVF2107[b]			
Low VEGF-A (*n*=192)	6.93	11.24	0.61 (0.43–0.87)
High VEGF-A (*n*=192)	5.52	9.10	0.59 (0.42–0.83)
E4599[c]			
Low VEGF-A (*n*=83)	5.59	6.57	0.71 (0.43–1.16)
High VEGF-A (*n*=83)	3.91	6.44	0.60 (0.36–0.99)
AVAiL (low-dose)[d]			
Low VEGF-A (*n*=301)	6.44	7.03	0.74 (0.59–0.94)
High VEGF-A (*n*=291)	5.95	6.67	0.76 (0.59–0.96)
AVAiL (high-doses)[d]			
Low VEGF-A (*n*=283)	6.44	7.03	0.89 (0.70–1.13)
High VEGF-A (*n*=303)	5.95	6.47	0.82 (0.65–1.04)
AVOREN[e]			
Low VEGF-A (*n*=192)	7.43	12.94	0.56 (0.40–0.78)
High VEGF-A (*n*=192)	3.81	7.72	0.74 (0.53–1.02)
OS			
AVF2107[b]			
Low VEGF-A (*n*=192)	17.97	24.57	0.75 (0.53–1.06)
High VEGF-A (*n*=192)	12.88	15.90	0.71 (0.52–0.97)
E4599[c]			
Low VEGF-A (*n*=83)	10.48	13.88	0.77 (0.49–1.23)
High VEGF-A (*n*=83)	8.54	10.84	0.84 (0.52–1.34)
AVAiL (low-dose)[d]			
Low VEGF-A (*n*=301)	15.80	14.88	0.99 (0.74–1.32)
High VEGF-A (*n*=291)	10.55	13.04	0.84 (0.64–1.10)
AVAiL (high-doses)[d]			
Low VEGF-A (*n*=283)	15.80	15.77	1.01 (0.76–1.36)
High VEGF-A (*n*=303)	10.55	13.01	0.95 (0.73–1.24)
AVOREN[e]			
Low VEGF-A (*n*=192)	28.42	34.56	0.73 (0.50–1.06)
High VEGF-A (*n*=192)	15.28	18.20	0.81 (0.58–1.13)



[a]Cytokine therapy (i.e., interferon alfa-2a), not chemotherapy, was used in AVOREN.

[b]The multivariate Cox model also includes ECOG PS (0 vs. 1), number of metastatic disease sites (1 vs. >1), location of primary tumor (colon vs. rectum).

[c]The multivariate Cox model also includes tumor measurability (yes vs. no), prior radiotherapy (yes *v* no), weight loss (<5% vs. >5%), and disease stage (IIIB vs. IV). The control arm in E4599 did not include a placebo.

[d]The multivariate Cox model also includes disease stage, sex, ECOG PS, and geographical region.

[e]The multivariate Cox model also includes geographical region (Western Europe, Eastern Europe, and other) and Motzer score (favorable, intermediate, poor).

Figure 3. HR for PFS and OS by VEGF-A level (defined at the median) for patients in the AVF2107, E4599, AVAiL, and AVOREN trials. BV, bevacizumab; Chemo, chemotherapy; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; PL, placebo; VEGF-A, vascular endothelial growth factor.

samples in each of the 4 phase III trials (Table 2). Point estimates for median OS showed a significant prognostic effect for circulating VEGF-A in all but 1 of the trials. In contrast, the prognostic effect of circulating VEGF-A levels on PFS did not reach statistical significance in any of the trials.

The predictive value of VEGF-A was assessed by calculating HR for PFS and OS, according to baseline total circulating VEGF-A level (as defined at the median) and treatment arm (see Fig. 3). For this analysis, the low-dose [7.5 mg/kg every 3 weeks (q3w)] and high-dose (15 mg/kg q3w) bevacizumab treatment groups in AVAiL were considered separately. In all but a single instance, the estimated HR for PFS and OS were less than 1 (range, 0.56–1.01) for bevacizumab-treated patients compared with placebo-treated patients across both VEGF-A subgroups. For patients in the low-plasma and high-plasma VEGF-A subgroups within each study, HR for PFS were generally similar, with overlapping CI. No clinically meaningful differences in HR for OS were evident according to baseline plasma VEGF-A levels.

For patients in AVOREN, total circulating VEGF-A levels at baseline were plotted against 2 established prognostic factors in mRCC: Motzer score and Karnofsky performance status. No significant correlation with either was observed (Supplementary Fig. S3).

Discussion

While there has been considerable debate over the role of circulating VEGF-A as a predictive marker for the use of bevacizumab, comprehensive analyses of multiple trials using a standardized methodology have only recently been conducted. Given that existing individual clinical trials are not sufficiently powered for biomarker subset analyses, we conducted this exploratory analysis to identify consistent trends in biomarker association with bevacizumab efficacy across multiple trials. This approach sought to overcome some of the deficiencies of earlier studies by using a qualified assay and detection methods that assess multiple epitopes, by ensuring sufficient patient numbers and statistical power, and by maintaining conformity to REMARK.

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This analysis of approximately 1,800 patients has yielded important new data on the prevalence of circulating VEGF-A levels in the plasma of patients with mCRC, NSCLC, and mRCC. Total circulating levels of VEGF-A ranged from less than 12.5 to more than 900 pg/mL in patients in the phase III studies, with a similar distribution regardless of cancer type. In contrast, circulating VEGF-A levels did not exceed 49 pg/mL in 40 healthy volunteers. In view of the high expression levels of VEGF-A mRNA noted in mRCC tumors relative to other tumors (2), the consistent distributions of circulating VEGF-A levels across tumor types suggest that baseline circulating VEGF-A levels in patients with metastatic disease do not directly correlate with primary tumor VEGF-A levels.

To evaluate a prognostic effect of circulating VEGF-A, outcomes in placebo-treated patients from phase III trials were analyzed in subsets defined by median circulating VEGF-A level. Median PFS and OS values in patients in the low VEGF-A group exceeded those of patients in the high VEGF-A group in all trials, and statistical significance was achieved for OS in 3 of the phase III trials (AVF2107, AVAiL, and AVOREN) in the overall patient population. In addition to median levels, additional cutoffs evaluating the first and last quartile of plasma VEGF distribution, that is, lowest 25% versus highest 25% provided equivalent results (33). These observations suggest that circulating VEGF-A has a prognostic effect and are consistent with other reports in the literature (10, 11, 34).

Multivariable analyses were conducted to evaluate the predictive significance of plasma VEGF-A levels on the treatment effect of bevacizumab. The analyses revealed that estimated HR for PFS were less than 1 (range, 0.56–0.89) for bevacizumab-treated versus placebo-treated patients in each study and reached at least a trend for significance in most instances (except for PFS in the low VEGF-A subgroup in E4599 and AVAiL). Similarly, estimated HR for OS with bevacizumab treatment were less than 1 in all but 1 instance (range, 0.71–1.01). This suggests that improvements in PFS and/or OS that were conferred by bevacizumab-containing treatment are independent of circulating VEGF-A levels at baseline.

In support of these findings, recent data using a novel VEGF-A ELISA assay with higher sensitivity to shorter, more soluble VEGF-A isoforms, including $VEGF_{110}$ and $VEGF_{121}$, also identified that baseline plasma VEGF-A had prognostic, but not predictive, value in mCRC, NSCLC, and mRCC (34). In contrast, this assay was predictive in determining bevacizumab response in metastatic breast cancer, gastric cancer, and pancreatic cancer (34, 35). High-baseline plasma VEGF-A was found to correlate with trends toward improved OS and PFS in these latter tumor types. There are several important differences, however, in the conduct of these analyses. In addition to the tumor types evaluated, samples in breast, gastric, and pancreatic cancers used EDTA plasma rather than citrated plasma (34). We used citrated plasma in the current analysis because this method of anticoagulation has minimal effect on platelet activation. Moreover, the low dynamic range of plasma VEGF-A levels in healthy controls (<12.5–49 pg/mL) suggests that the impact of uncontrolled platelet activation would be minimal in patients with cancer (<12.5–>900 pg/mL; see Fig. 1). One caveat is that the number of platelets and levels of VEGF isoforms in platelets at baseline in patients with cancer may be different from healthy donors. It is unclear whether isoforms of VEGF-A are differentially represented in citrated versus EDTA plasma or whether platelets release different isoforms that affect treatment efficacy in some indications, thus confounding comparisons between these studies. To date, data are not available to determine whether circulating VEGF-A using EDTA plasma is predictive in mCRC, NSCLC, and mRCC; however, the number of patients evaluated and the determination of prognostic significance for circulating VEGF-A in these tumor types lend credence to the validity of the current findings.

Limitations of the current analysis include its retrospective nature, differing sampling dates between archival tissue and matched baseline plasma samples, the use of a single measurement time point, the analysis cutoffs used to dichotomize this continuous biomarker, and the limitations of the assay used. In addition, any conclusions about the predictive ability of total circulating VEGF-A must be viewed within the context of the specific cancer type and chemotherapy (AVF2107, E4599, AVAiL) or immunotherapy (AVOREN) regimen with which bevacizumab was combined. Given that VEGF-A is a dynamic target, the use of archival tumors to assess tumor VEGF-A expression could also confound any correlations to the assessment of plasma VEGF-A at study start. Additional studies evaluating assays that recognize specific isoforms of VEGF-A are currently ongoing and may clarify what, if any, role circulating VEGF-A has as a predictive biomarker for bevacizumab-based treatment in cancer.

Disclosure of Potential Conflicts of Interest

A.M. Jubb has ownership interest (including patents) in Genentech/Roche. D. Chen is a consultant/advisory board member for Statistical Consulting. G. Meng has ownership interest (including patents) in Roche stock. S. Scherer is employed (other than primary affiliation; e.g., consulting) by Genetech as a Global Biomarker head Oncology. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: D. Chen, C. Bernaards, S. Scherer, D.S. Chen
Development of methodology: P.S. Hegde, A.M. Jubb, D. Chen, N.F. Li, G. Meng, S. Scherer, D.S. Chen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.M. Jubb, D.S. Chen
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.M. Jubb, D. Chen, N.F. Li, C. Bernaards, R. Elliott, S. Scherer, D.S. Chen
Writing, review, and/or revision of the manuscript: P.S. Hegde, A.M. Jubb, D. Chen, N.F. Li, G. Meng, C. Bernaards, R. Elliott, S. Scherer, D.S. Chen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Bernaards, D.S. Chen
Study supervision: S. Scherer, D.S. Chen

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Priti S. Hegde, Adrian M. Jubb, Dafeng Chen, et al.

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