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Biomarker results from the AVADO phase 3 trial of first-line bevacizumab plus docetaxel for HER2-negative metastatic breast cancer

D W Miles^{*,1,11}, S L de Haas^{2,11}, L Y Dirix³, G Romieu⁴, A Chan⁵, X Pivot⁶, P Tomczak⁷, L Provencher⁸, J Cortés⁹, P R Delmar² and S J Scherer¹⁰

¹Mount Vernon Cancer Centre, Rickmansworth Road, Northwood, London, HA6 2RN, UK; ²F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124, 4070 Basel, Switzerland; ³St Augustinus Hospital, Oosterveldlaan 24, 2610 Wilrijk-Antwerp, Belgium; ⁴Centre Régionale de Lutte contre le Cancer, Val d'Aurelle, Rue Croix Verte, 34298 Montpellier, France; ⁵Mount Hospital, Mount Breast Group, 150 Mounts Bay Road, Perth, WA 6000, Australia; ⁶Centre Hospitalier Universitaire de Besançon, 25030 Besançon, France; ⁷Klinika Onkologii Oddzial Chemioterapii, 60-569 Poznań, Poland; ⁸Centre des Maladies du Sein Deschênes-Fabia, Québec, G1S 4L8, Canada; ⁹University Hospital Vall d'Hebron, Passeig Vall d'Hebron, 119-129, 08035 Barcelona, Spain and ¹⁰Genentech Inc., 1 DNA Way, San Francisco, CA 94080, USA

Background: Combining bevacizumab with first-line chemotherapy significantly improves progression-free survival (PFS) in HER2-negative metastatic breast cancer (mBC). However, identification of patients benefitting most from bevacizumab remains elusive. The AVADO trial included an extensive optional exploratory biomarker programme.

Methods: Patients with HER2-negative mBC were randomised to receive docetaxel with placebo or bevacizumab. The primary end point was PFS. Plasma samples were analysed using a multiplex ELISA. Blood mRNA expression was assessed using quantitative PCR. Tumour tissue samples were analysed by immunohistochemistry. Single-nucleotide polymorphisms (SNPs) involved in the VEGF pathway were analysed in germline DNA.

Results: Samples for biomarker analysis were available from 24–54% of the 736 treated patients (depending on specimen type). The most consistent potential predictive effect was observed with plasma VEGF-A and VEGFR-2; high baseline concentrations were associated with greater treatment effect. Blood mRNA analyses suggested a greater bevacizumab effect in patients with high VEGF₁₂₁. No consistent predictive effect was seen for tumour neuropilin or other candidate tumour markers by immunohistochemistry, or for any of the SNPs investigated.

Conclusion: Plasma VEGF-A and VEGFR-2 are potential predictive markers for bevacizumab efficacy, supporting findings in gastric and pancreatic cancers. Plasma VEGF-A is being evaluated prospectively in mBC in the MERiDiAN trial.

The humanised monoclonal antibody bevacizumab, which targets all isoforms of vascular endothelial growth factor A (VEGF-A), significantly improves progression-free survival (PFS) when combined with first- or second-line chemotherapy for HER2-negative locally recurrent/metastatic breast cancer (LR/mBC) (Gray *et al*, 2009; Miles *et al*, 2010a; Brufsky *et al*, 2011; Robert

et al, 2011). The magnitude of the benefit shows some variation between the first-line LR/mBC trials, with the most marked effect observed in the E2100 trial evaluating bevacizumab combined with weekly paclitaxel and a more modest, but nevertheless significant, PFS improvement seen in the AVADO trial evaluating bevacizumab combined with docetaxel.

*Correspondence: Dr DW Miles; E-mail: david.miles@doctors.org.uk

¹¹These authors contributed equally to this work.

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Numerous subgroup analyses have been conducted in an effort to identify those patients deriving the most substantial benefit from bevacizumab (O'Shaughnessy *et al*, 2009, 2010a, 2010b; Miles *et al*, 2010b). However, the relative treatment effect of bevacizumab appears to be similar in all subgroups identified by traditional clinical characteristics. Consequently, patient selection remains a challenge.

Most of the phase 3 bevacizumab clinical trials across tumour types include extensive biomarker evaluation. Of the various markers evaluated, circulating VEGF-A has been the primary focus to date. VEGF-A is the target of bevacizumab and plays a crucial role in the stimulation of angiogenesis via signalling through VEGF receptor 2 (VEGFR-2) (Ferrara, 2004). Initial biomarker analyses of bevacizumab trials in metastatic colorectal, renal, and non-small-cell lung cancers indicated that VEGF-A was a prognostic marker but had no predictive value (Hegde *et al*, 2013). Patients with high plasma VEGF-A concentrations had a poor prognosis, irrespective of treatment administered, but plasma VEGF-A concentrations provided no predictive information on the likely benefit of bevacizumab.

The phase 3 E2100 trial in LR/mBC included biomarker analyses and reported a correlation between two single-nucleotide polymorphisms (SNPs) in VEGF-A (−2578 and −1154) and overall survival (OS) in patients receiving bevacizumab (Schneider *et al*, 2008). However, no such correlation was seen between these SNPs and PFS. Additional analysis of VEGF-A and VEGFR-2 expression assessed by immunohistochemistry (IHC) revealed no correlations with outcome.

A retrospective analysis of tumour tissue samples from the AVF2119g trial of capecitabine with or without bevacizumab in heavily pretreated patients with LR/mBC showed a weak prognostic but not a predictive effect of tumour VEGF-A expression (Jubb *et al*, 2011). However, low expression of neuropilin-1, thymidine phosphorylase, VEGF-C, and delta-like ligand 4 showed trends towards increased PFS benefit with bevacizumab (Jubb *et al*, 2011). Similar findings for neuropilin-1 have been reported in two trials of bevacizumab in gastric cancer (AVAGAST) (Ohtsu *et al*, 2011) and colorectal cancer (NO16966) (Saltz *et al*, 2008), in which low neuropilin expression was associated with greater bevacizumab treatment effect (Foerzler *et al*, 2010; Van Cutsem *et al*, 2012).

Additional candidate markers have emerged from the extensive bevacizumab biomarker programme in trials across a broad range of tumour types. Analysis of DNA samples from the double-blind randomised AViTA trial in pancreatic cancer (Van Cutsem *et al*, 2009) and AVOREN trial in renal cell cancer (Escudier *et al*, 2007) suggested a correlation between the rs9582036-A allele in VEGFR-1 and improved OS (in AViTA) and PFS (both trials) in patients receiving bevacizumab-containing therapy, but not in those treated with chemotherapy alone, indicating potential predictive value (Lambrechts *et al*, 2012).

Here we report findings from analyses of plasma, tumour, DNA, and RNA samples from the AVADO trial.

MATERIALS AND METHODS

The AVADO trial was a double-blind, placebo-controlled, randomised phase 3 trial evaluating bevacizumab in combination with docetaxel as first-line therapy for HER2-negative LR/mBC. Full details have been published previously (Miles *et al*, 2010a). Patients with HER2-negative LR/mBC who had received no previous chemotherapy for advanced disease were randomised 1:1:1 to receive docetaxel 100 mg m^{−2} every 3 weeks with either placebo, bevacizumab 7.5 mg kg^{−1}, or bevacizumab 15 mg kg^{−1} every 3 weeks. Docetaxel was continued for up to nine cycles at the

investigator's discretion; bevacizumab or placebo was continued until disease progression. Patients initially randomised to placebo were allowed to cross over to bevacizumab at progression (or at study unblinding). All patients were allowed to receive bevacizumab in combination with their second-line chemotherapy. Patients and clinicians were blinded to treatment assignment.

The primary end point was PFS. Secondary end points included overall response rate, OS, and safety. Potential biomarkers in plasma, tumour tissue, and blood (RNA and DNA) involved in angiogenesis and tumourigenesis were evaluated for exploratory purposes. Participation in the biomarker element of the trial was optional and required separate written informed consent.

The protocol was approved at each participating site by an independent ethics committee or institutional review board. The trial was carried out in accordance with the Declaration of Helsinki. All patients provided written informed consent before study entry.

Sample collection and analysis. A 5 ml blood sample in EDTA for plasma analysis was obtained at baseline (after randomisation but before the first dose of study drug) and at the time of disease progression. Plasma samples were analysed centrally at Roche Diagnostics Ltd (Penzberg, Germany) using immunological multiparametric chip technique, a Roche proprietary multiplex enzyme-linked immunosorbent assay (ELISA) platform. This ELISA included VEGF-A, VEGFR-1, VEGFR-2, and E-selectin. For VEGF-A, the assay differs from that used in earlier biomarker analyses of bevacizumab, having greater sensitivity for shorter vs longer isoforms of VEGF-A. Intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 were evaluated using separate single ELISAs (R&D Systems, Minneapolis, MN, USA).

Historical paraffin-embedded blocks of primary tumour tissue (or metastatic lesions if the primary tumour was not available) were collected and used to create tissue microarrays and tissue sections for DNA and RNA isolation and analysis. Immunohistochemistry analysis was performed at HistoGeneX (Antwerp, Belgium) (Supplementary Table 1). Tumour-derived RNA was used to evaluate expression levels of VEGF-A, VEGF-C, VEGF-D, VEGFR-1, VEGFR-3, placental growth factor, and neuropilin-1 in reference to the *G6PDH* housekeeping gene. A quantitative reverse transcriptase-PCR (qRT-PCR) was performed on LightCycler 480 (Roche Molecular Systems, Penzberg, Germany) using research assays developed in-house (Roche Applied Science, Penzberg, Germany).

A 5 ml blood sample was collected in EDTA to extract germline DNA. A total of 26 SNPs involved in angiogenesis and tumourigenesis were investigated using kinetic PCR and sequencing. These SNPs were selected based on previous findings and included polymorphisms involved in the VEGF pathway (Supplementary Table 2).

Blood samples for mRNA expression profiling were collected in PAXgene vacutainers (two samples of 2.5 ml) and qRT-PCR analysis was performed on the LightCycler 480 instrument (Roche Molecular Systems) after RNA had been extracted. Analysis included qRT-PCR using research assays developed in-house (Roche Molecular Sciences, Pleasanton, CA, USA) of the following VEGF-A isoforms: VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₉, and VEGF₂₀₆.

Both SNP and qRT-PCR analyses (tumour and blood derived) were performed at Roche Translational Research Laboratories (Basel, Switzerland).

Statistical analyses. The statistical analyses for the translational research substudy are strictly exploratory in nature. For analyses of plasma, tumour tissue H score, and mRNA expression in tumour tissue and blood samples, continuous biomarker variables were dichotomised as low or high for each patient using the median value as the prespecified cutoff. Candidate plasma biomarker concentrations were also categorised into quartiles for further

descriptive subgroup analyses. All pretreatment values were correlated with PFS (before start of subsequent antineoplastic therapy) using simple and multiple Cox regression approaches.

SNPs were analysed in samples from Caucasian patients. Samples from non-Caucasian patients were excluded as a precautionary measure to minimise genetic variability. Genotypes were grouped by allelic coding (0, 1, or 2). Simple Cox regression with no adjustments for multiple testing or baseline prognostic factors was performed to correlate genotypes with PFS and OS.

To assess the potential predictive value of the biomarkers, subgroup analyses were performed and treatment by biomarker interaction terms were tested in the Cox regression model. The Cox model for interaction testing also included stratification factors (oestrogen and progesterone hormone receptor status, measurable disease, and prior adjuvant taxane therapy) as covariates except in the genetic analysis. As the interaction test is acknowledged to have low power, a trend may be indicated by a *P*-value marginally exceeding the 5% threshold.

Unless otherwise specified (e.g., in the genetics data analysis), hazard ratios (HRs) refer to the comparison of one bevacizumab arm with the control arm. The Kruskal–Wallis test was used for exploratory analysis of potential correlations between VEGF-A SNPs and baseline plasma VEGF-A concentrations.

Formal *P*-value correction for multiple testing was not applied, but the multiplicity was taken into account in the interpretation of results. This approach was considered the most suitable given the exploratory nature of the analyses and considering that the measured biomarkers were selected based on biological relevance. In addition, each category of biomarker was measured in different subsets of patients, and consequently comparisons between categories of biomarkers are not possible.

RESULTS

Patient population. Plasma samples were available from 396 (54%) of the 736 patients treated in AVADO. Tumour samples for IHC analysis were available from 176 patients (24%). DNA samples for analysis of SNPs were available from 336 Caucasian patients (46% of the overall population).

Generally, the biomarker analysis populations were representative of the overall population (Table 1 and Supplementary Table 3). However, there was typically a higher proportion of Caucasian patients in biomarker analysis populations because some Asian countries did not participate in the biomarker sampling and non-Caucasian samples were excluded from the DNA analysis. PFS and OS in the biomarker populations were consistent with those in the overall population (Table 1).

Analysis of plasma samples. The HR point estimate favoured the bevacizumab arms in all subgroups except for patients with low VEGFR-2 concentrations receiving bevacizumab 7.5 mg kg⁻¹ (Figure 1). The most pronounced and consistent potential predictive effect was seen for VEGF-A and VEGFR-2. In general, higher plasma concentrations of VEGF-A and VEGFR-2 were associated with greater treatment effect (Figures 2–4). In addition, VEGF-A showed prognostic value: in placebo-treated patients, PFS was worse in those with high vs low VEGF-A concentrations. Concentrations of VEGF-A and VEGFR-2 did not appear to differ between clinically defined subgroups (e.g., Eastern Cooperative Oncology Group performance status, hormonal receptor status, and tumour burden assessed by the sum of largest diameters). A multiple Cox regression model was run with randomisation stratification factors (oestrogen and progesterone hormone receptor status, measurable disease, and prior adjuvant taxane therapy) as covariates to correct for baseline characteristics. Using this model, there was a suggestion that concentrations of VEGF-A and VEGFR-2 had predictive value after accounting for other baseline characteristics (Figure 1). For VEGF-A, the interaction *P*-value was 0.0136 for the bevacizumab 7.5 mg kg⁻¹ arm and 0.0808 for the bevacizumab 15 mg kg⁻¹ arm. For VEGFR-2, the interaction *P*-values were 0.0342 and 0.2545, respectively. Correlation analyses revealed no relationship between baseline characteristics and either VEGF-A or VEGFR-2 (Table 2).

The median concentration of VEGF-A was prespecified as the initial cutoff for the analysis. The HR of 0.49 in the high VEGF-A subgroup indicated a substantial clinical benefit (Figure 2). Further exploration of the predictive value of VEGF-A was undertaken by subdividing the bevacizumab 15 mg kg⁻¹ and placebo arms into quartiles by VEGF-A concentration. The point estimates of the quartiles showed a consistent improvement in the HR with

Table 1. Baseline characteristics and efficacy results in the plasma biomarker population compared with the overall study population

Value	Plasma biomarker population			Overall study population		
	Placebo (n = 129)	Bevacizumab 7.5 mg kg ⁻¹ (n = 129)	Bevacizumab 15 mg kg ⁻¹ (n = 138)	Placebo (n = 241)	Bevacizumab 7.5 mg kg ⁻¹ (n = 248)	Bevacizumab 15 mg kg ⁻¹ (n = 247)
Median age, years (range)	55 (30–83)	54 (29–83)	54 (32–75)	55 (29–83)	54 (26–83)	55 (27–76)
ECOG PS 0, %	65	63	62	62	61	61
Caucasian, %	94	95	96	83	84	84
≥3 Lesions, %	39	50	51	41	49	49
ER and PgR negative, %	21	22	19	22	21	23
Hazard ratio for PFS (95% CI) ^a		0.72 (0.53–0.98)	0.67 (0.49–0.90)		0.69 ^b (0.54–0.89)	0.61 ^b (0.61–0.78)
Median PFS, months	7.8	8.7	8.6	8.0	8.7	8.8
Hazard ratio for OS (95% CI) ^a		1.06 (0.74–1.51)	1.06 (0.75–1.51)		1.05 (0.81–1.36)	1.03 (0.70–1.33)
Median OS, months	31.9	32.8	31.3	31.9	30.8	30.2

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; ER = oestrogen receptor; OS = overall survival; PFS = progression-free survival; PgR = progesterone receptor.

^aDatabase snapshot date differs between PFS (31 October 2007) and OS (30 April 2009) data but matches between the biomarker population and the overall study population.

^bStratified analysis.

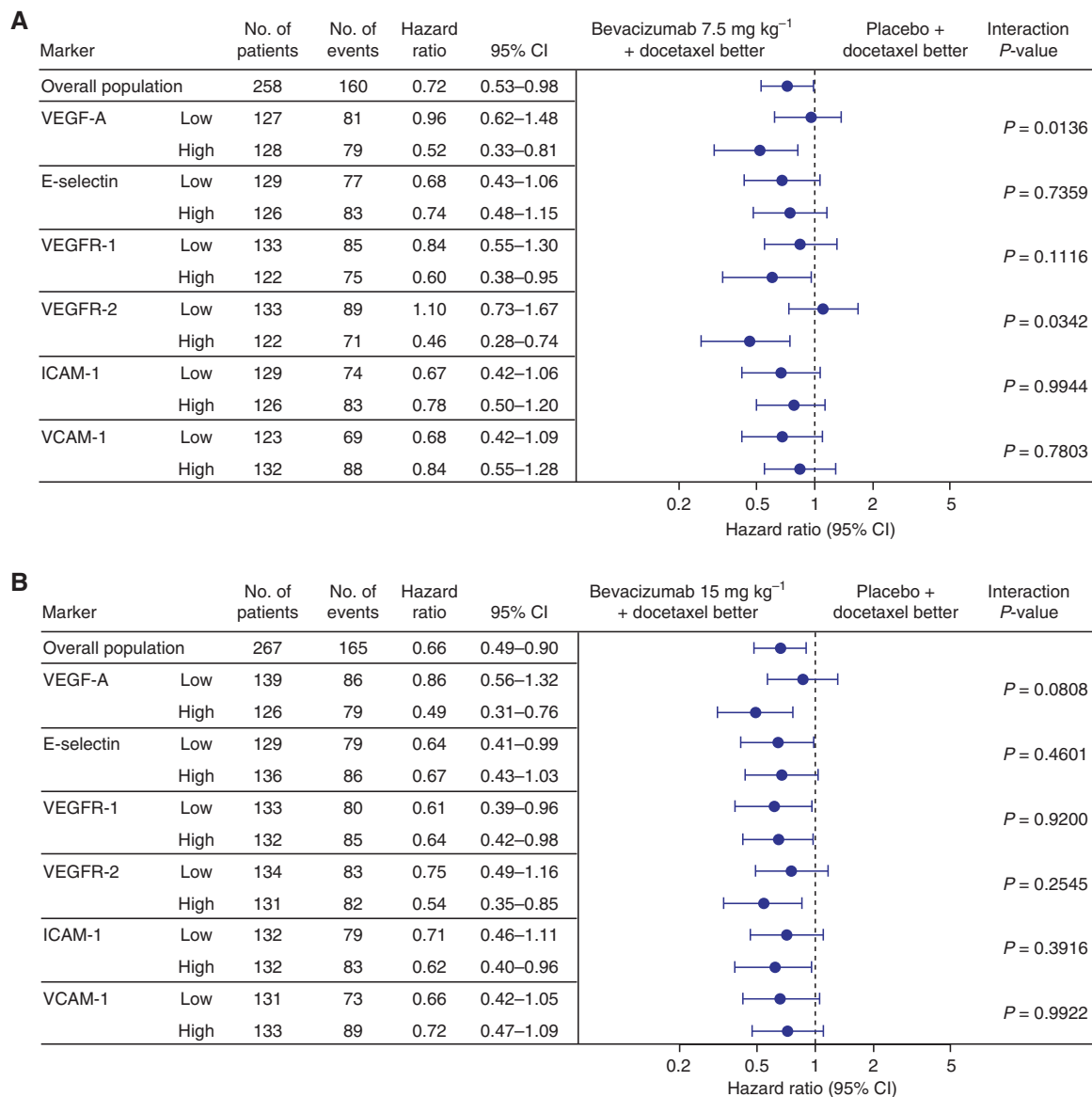


Figure 1. Progression-free survival (PFS) by plasma biomarker concentration. (A) Bevacizumab 7.5 mg kg⁻¹ vs placebo and (B) bevacizumab 15 mg kg⁻¹ vs placebo. Hazard ratio shows the comparison of bevacizumab vs placebo. Median cutoff values: VEGF-A = 125.0 pg ml⁻¹; VEGFR-1 = 70.3 pg ml⁻¹; VEGFR-2 = 11.0 pg ml⁻¹; E-selectin = 35.7 ng ml⁻¹; VCAM-1 = 577.0 ng ml⁻¹; ICAM-1 = 223.0 ng ml⁻¹. ICAM-1 = intracellular adhesion molecule 1; VCAM-1 = vascular cell adhesion molecule 1; VEGF-A = vascular endothelial growth factor A; VEGFR-1 = vascular endothelial growth factor receptor 1.

increasing VEGF-A concentrations, although the 95% confidence interval (CIs) for all quartiles overlapped (Figure 3B). The HR for PFS was 0.87 (95% CI 0.47–1.59) in the first quartile vs 0.40 (95% CI 0.20–0.79) in the highest quartile, and the difference in median PFS was more pronounced than in the other quartiles. The HR of 0.55 in the third quartile also suggested a substantial effect. The analysis by quartile in the 7.5 mg kg⁻¹ arm showed a less pronounced trend (Figure 3A).

None of the other markers explored showed a particularly pronounced difference between low and high concentrations in plasma samples. For ICAM-1, the PFS benefit was numerically greater with low marker concentrations in the bevacizumab 7.5 mg kg⁻¹ arm and greater with high marker concentrations in the bevacizumab 15 mg kg⁻¹ arm.

Currently, no data are available from samples collected at the time of disease progression. Although it will be important to analyse changes in potential predictive markers over time,

interpretation is complicated by the considerable challenges in measuring VEGF-A in the presence of bevacizumab.

Analysis of blood and tumour mRNA. Analysis of blood mRNA suggested a greater bevacizumab treatment effect on PFS in patients with high VEGF₁₂₁ (interaction P-value 0.0367) when comparing the 15 mg kg⁻¹ dose with placebo. The difference was less pronounced in the 7.5 mg kg⁻¹ dose group (interaction P-value 0.076). No predictive effect was seen for either VEGF₁₆₅ or VEGF₁₈₉.

There was no correlation between plasma protein VEGF-A concentrations and blood VEGF₁₂₁ mRNA concentrations. None of the tumour mRNA markers analysed showed potential predictive value (data not shown).

Immunohistochemistry analysis of tumour samples. In the 15 mg kg⁻¹ arm, high (>median) neuropilin H score was associated with greater benefit from bevacizumab (HR 0.90 for

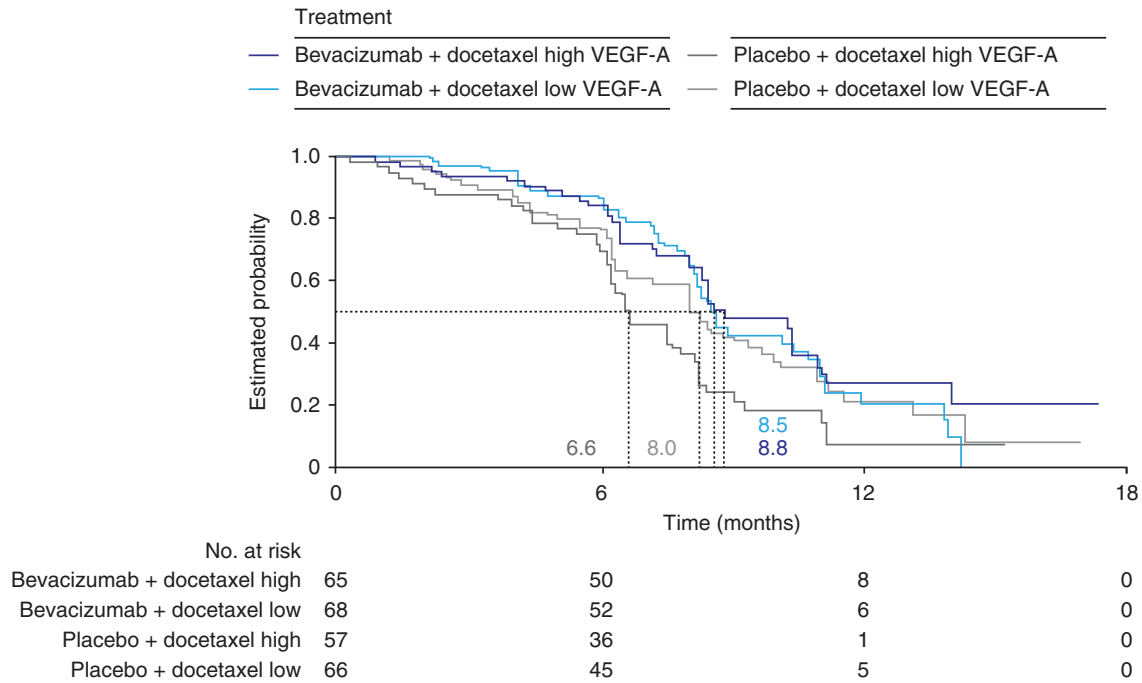


Figure 2. Plasma vascular endothelial growth factor A (VEGF-A) concentrations: progression-free survival.

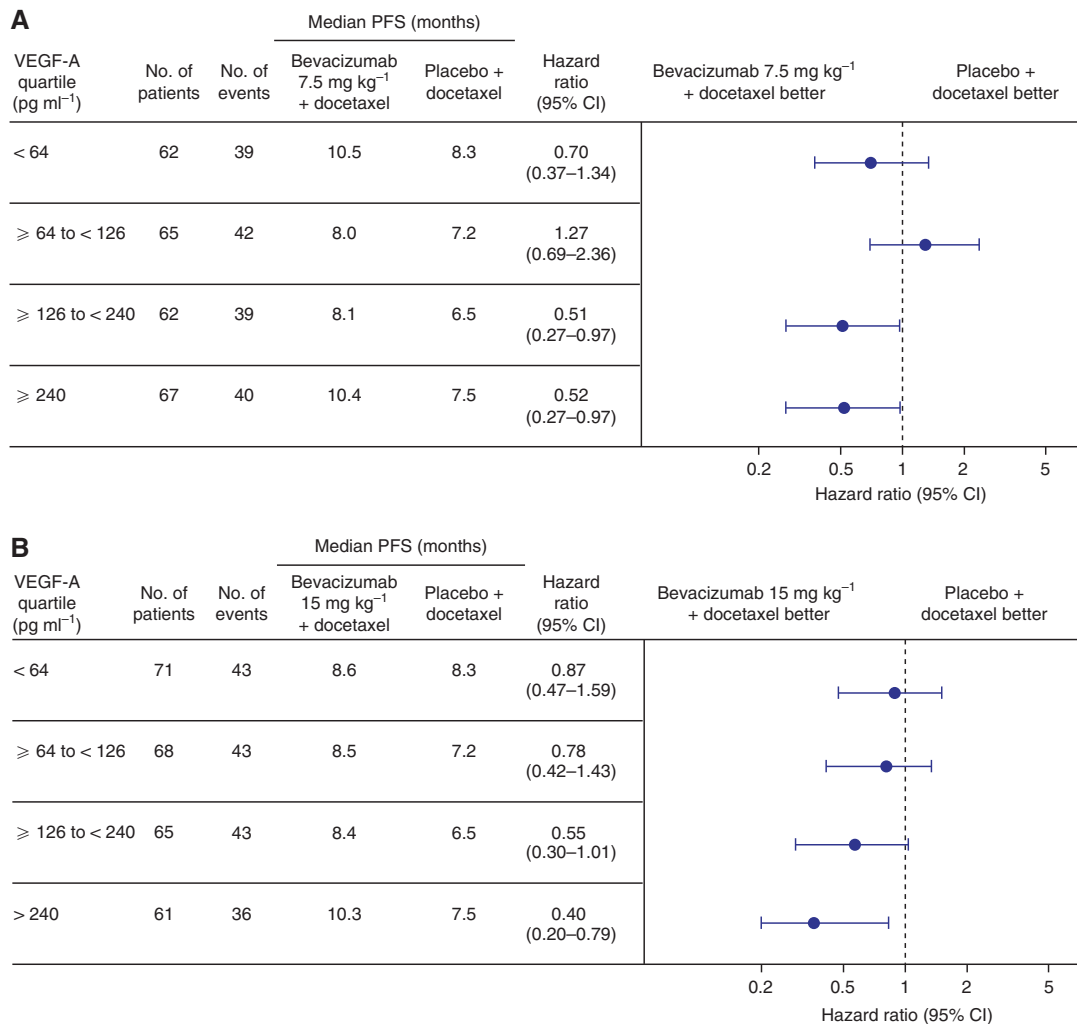


Figure 3. Plasma vascular endothelial growth factor A (VEGF-A) concentrations by quartile. (A) Bevacizumab 7.5 mg kg⁻¹ vs placebo and (B) bevacizumab 15 mg kg⁻¹ vs placebo.

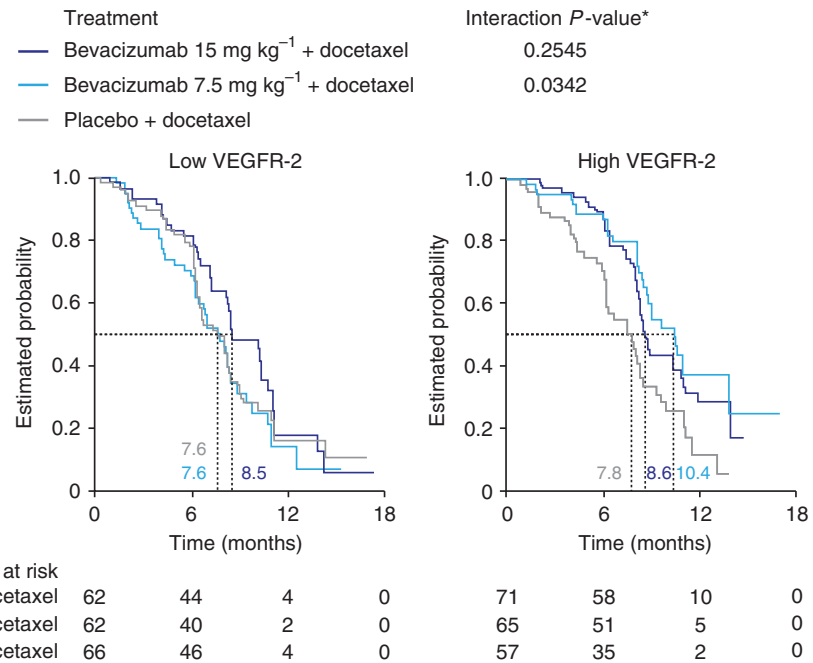


Figure 4. Progression-free survival by plasma vascular endothelial growth factor receptor 2 (VEGFR-2) level. In the low VEGFR-2 subgroup, hazard ratios were 1.10 (95% CI 0.72–1.67) for bevacizumab 7.5 mg kg⁻¹ vs placebo (n = 133) and 0.76 (95% CI 0.49–1.17) for bevacizumab 15 mg kg⁻¹ vs placebo (n = 134). In the high VEGFR-2 subgroup, hazard ratios were 0.47 (95% CI 0.29–0.75) for bevacizumab 7.5 mg kg⁻¹ vs placebo (n = 122) and 0.55 (95% CI 0.35–0.85) for bevacizumab 15 mg kg⁻¹ vs placebo (n = 131). Wald test. Cox regression factors: treatment, biomarker level, binary stratification factors, interaction term of treatment by biomarker level. *Wald test. Cox regression factors: treatment, biomarker level, binary stratification factors, interaction term of treatment by biomarker level.

Table 2. Correlation between baseline characteristics and VEGF-A and VEGFR-2 concentrations (all arms pooled)

	VEGF-A			VEGFR-2		
	Low, n (%) (n = 197)	High, n (%) (n = 196)	Interaction P-value	Low, n (%) (n = 197)	High, n (%) (n = 196)	Interaction P-value
Age, years						
<65	157 (80)	156 (80)	1.0000	149 (76)	164 (84)	0.0599
≥65	40 (20)	40 (20)		48 (24)	32 (16)	
ECOG PS						
0	125 (63)	120 (61)	0.6739	118 (60)	127 (65)	0.4616
1	68 (35)	74 (38)		74 (38)	68 (35)	
2	1 (<1)	0		1 (<1)	0	
Disease-free interval						
≤median	67 (34)	70 (36)	0.8320	70 (36)	67 (34)	0.9156
>median	128 (65)	125 (64)		127 (64)	126 (64)	
ER status						
Negative	49 (25)	54 (28)	0.5662	56 (28)	47 (24)	0.3604
Positive	148 (75)	140 (71)		141 (72)	147 (75)	
ER/PgR combined						
Negative	40 (20)	40 (20)	1.0000	48 (24)	32 (16)	0.0600
Positive	157 (80)	155 (79)		149 (76)	163 (83)	
No. of metastatic sites						
≥3	87 (44)	95 (48)	0.5425	94 (48)	88 (45)	0.6119
<3	106 (54)	101 (52)		101 (51)	106 (54)	

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group performance status; ER = oestrogen receptor; PgR = progesterone receptor; VEGF-A = vascular endothelial growth factor A; VEGFR-2 = vascular endothelial growth factor receptor 2.

Table 3. Correlation between SNPs and PFS by treatment arm

SNP	Placebo			Bevacizumab 7.5 mg kg ⁻¹				Bevacizumab 15 mg kg ⁻¹			
	Events/ n	Allelic HR ^a	Allelic P-value ^b	Events/ n	Allelic HR ^a	Allelic P-value ^b	Interaction P-value	Events/ n	Allelic HR ^a	Allelic P-value ^b	Interaction P-value
VEGF: c. +405/c.-634	89/103	1.503	0.027	98/115	0.934	0.647	0.046	91/109	0.994	0.972	0.087
VEGF: c. +936 C>T	89/103	0.937	0.763	99/116	1.026	0.902	0.813	91/109	1.472	0.039	0.128
VEGF: c.-1154 A>G	89/104	1.091	0.618	99/117	1.443	0.015	0.175	89/109	0.996	0.981	0.767
VEGF: c.-2578 C>A	88/102	0.727	0.043	99/116	1.184	0.237	0.018	91/109	0.924	0.605	0.229
VEGF: c.-460T>C; c.-1498T>C	89/104	0.750	0.066	99/118	1.190	0.213	0.024	88/108	0.925	0.624	0.312
VEGFR-1: rs9554316	88/102	0.992	0.965	99/115	0.945	0.771	0.835	91/109	1.072	0.687	0.774
VEGFR-1: rs9582036	89/103	0.952	0.764	99/116	0.953	0.752	0.980	91/109	1.104	0.552	0.565

Abbreviations: HR = hazard ratio; PFS = progression-free survival; SNP = single-nucleotide polymorphism; VEGF = vascular endothelial growth factor; VEGFR-1 = vascular endothelial growth factor receptor 1.

^aHazard ratio between SNPs within the treatment arm.

^bComparison within the treatment arm.

Values in bold represent *P*-values <0.05.

low neuropilin vs 0.35 for high neuropilin with the Santa Cruz antibody; interaction *P*-value 0.03). In the 7.5 mg kg⁻¹ arm, there was little difference between the subgroups when the Santa Cruz antibody was used. The reverse trend was seen using the R&D antibody (HR 0.47 for low neuropilin vs 1.06 for high neuropilin; interaction *P*-value 0.0461).

High VEGFR-1 expression appeared to be associated with greater PFS benefit from bevacizumab only in the 15 mg kg⁻¹ arm (HR 0.33 for high VEGFR-1 vs 0.89 for low VEGFR-1; interaction *P*-value 0.07). No correlation was observed between plasma VEGF-A concentration and either tumour IHC or mRNA VEGF-A. None of the other potential tumour markers explored showed predictive value (Supplementary Table 4).

Analysis of DNA samples. Results of SNP-based analyses showed no clear correlation between the SNPs evaluated and efficacy (Table 3). One of the SNPs evaluated in E2100 (-2578) showed a weak correlation with PFS in the placebo arm but not in the bevacizumab arms. Each additional C allele was associated with a 27% decrease in the risk of progression or death (allelic HR = 0.727), indicating a potential prognostic effect. In the bevacizumab 7.5 mg kg⁻¹ arm, there was an indication of potential treatment by genotype interaction (interaction *P* = 0.018) for the VEGF-A -2578 C/A polymorphism for PFS, but no correlation was seen between efficacy and this SNP in the bevacizumab 15 mg kg⁻¹ arm. The -1154 VEGF-A SNP showed a weak correlation with PFS in the bevacizumab 7.5 mg kg⁻¹ arm (allelic HR = 1.443) but not the bevacizumab 15 mg kg⁻¹ arm.

There was no clear correlation between efficacy and the VEGFR-1 rs9582036 SNP or any of the other SNPs evaluated (data not shown). In addition, exploratory analyses revealed no correlation between VEGF-A SNPs and baseline plasma VEGF-A concentrations (data not shown).

DISCUSSION

Biomarker analyses from the AVADO trial using a novel ELISA identified plasma VEGF-A and VEGFR-2 as potential candidates for predicting PFS in patients receiving bevacizumab-containing therapy for HER2-negative LR/mBC. Patients with high plasma VEGF-A concentrations at baseline appeared to benefit more from bevacizumab than those with lower VEGF-A concentrations.

Similarly, those with high VEGFR-2 concentrations at baseline appeared to derive a greater PFS benefit from bevacizumab than those with low VEGFR-2 concentrations. Neither of these markers correlated with clinical baseline characteristics, such as age, oestrogen receptor status, disease-free interval, or number of metastatic sites, suggesting that they have independent predictive value and are not, for example, surrogate markers for tumour burden or more aggressive disease.

Recently, similar results were reported suggesting predictive potential of plasma VEGF-A and VEGFR-2 using the same novel ELISA. In the AViTA trial (which evaluated gemcitabine/erlotinib with or without bevacizumab in patients with metastatic pancreatic cancer), biomarker analyses suggested a predictive role for both VEGF-A and VEGFR-2 (Van Cutsem *et al*, 2011). Likewise, in AVAGAST (which evaluated capecitabine/cisplatin with or without bevacizumab in patients with previously untreated metastatic gastric cancer), there were trends towards greater benefit (OS and PFS) with bevacizumab in patients with higher plasma VEGF-A concentrations. This effect was driven primarily by the subgroup of non-Asian patients (Van Cutsem *et al*, 2012). Biomarker results from three additional randomised phase 3 trials of bevacizumab (AVF2107g in colorectal cancer, AVOREN in renal cell cancer, and AVAiL in non-small-cell lung cancer) were inconclusive and did not confirm the potential predictive value of plasma VEGF-A for outcome with bevacizumab-containing therapy (Jayson *et al*, 2011). The apparent discrepancy may be attributable to differences in preanalytical factors and sample handling, the selected cutoff for dichotomisation of the study populations, or biological differences between the tumour types. Evaluation using fresh samples from recently completed trials is planned to try to understand whether the potential predictive value of plasma VEGF-A may apply to some but not all tumour types in which bevacizumab has demonstrated activity.

The observed stepwise effect in the quartile analysis of VEGF-A provides a reassuring signal of the potential predictive role of VEGF-A, indicating that, irrespective of the cutoff selected for categorisation of plasma biomarker concentrations, higher VEGF-A appears to be associated with greater PFS benefit with bevacizumab. The prognostic role of plasma VEGF-A is consistent with findings across tumour types (Jayson *et al*, 2011).

None of the other plasma markers explored showed a clear, consistent correlation with PFS. Intracellular adhesion molecule 1, which appeared to have predictive potential in non-small-cell lung cancer (Dowlati *et al*, 2008), showed an

opposite trend in the AVADO bevacizumab 7.5 and 15 mg kg⁻¹ treatment arms.

Immunohistochemistry analyses of tumour samples and RNA analyses revealed no potential predictive marker. Of note, low tumour neuropilin, which was suggested to have predictive value in colorectal cancer (Foerzler *et al*, 2010), gastric cancer (Van Cutsem *et al*, 2012), and heavily pretreated LR/mBC (Jubb *et al*, 2011), was associated with greater PFS benefit from bevacizumab in the AVADO trial, but this was observed only in the 7.5 mg kg⁻¹ arm with one of the two antibodies used. The opposite trend was observed in the 15 mg kg⁻¹ dose group using the Santa Cruz antibody. Overall, these results do not confirm previous findings for neuropilin. High VEGFR-1 expression appeared to be associated with greater PFS benefit only in the 15 mg kg⁻¹ dose group. A notable weakness of the tumour tissue analyses is the limited availability of samples (only 24%). Furthermore, only tissue microarrays were available for tumour tissue evaluation, enabling analysis of only a small part of the tumour, which may not be representative if the tumour is heterogeneous.

The results of SNP-based analyses of the AVADO trial did not confirm the previously reported correlations between SNPs and efficacy in the E2100 (Schneider *et al*, 2008), AVITA, and AVOREN (Lambrechts *et al*, 2012) trials. Furthermore, none of the other SNPs evaluated correlated with efficacy. We detected no clear correlation between VEGF-A SNPs and either efficacy or baseline plasma VEGF-A concentrations. The potential of SNPs as predictive markers for both efficacy and tolerability (hypertension) continues to be explored in trials across tumour types (Lambrechts *et al*, 2011a, 2011b).

The major limitations of these analyses include the small sample sizes, particularly for the IHC analyses, and the retrospective exploratory nature of the translational research. As specific biomarker hypotheses were not prespecified and multiple analyses were performed, the risk of false-positive results cannot be overlooked. Pooling the two bevacizumab arms may have increased the power but potentially introduces other complexities for interpretation because of the unclear dose effect for bevacizumab in breast cancer. Notwithstanding these challenges, the results can be interpreted in the context of hypotheses generated from other trials in the bevacizumab biomarker programme, and based on biological relevance.

Although tumour tissue and RNA and DNA analyses yielded no clear candidates for a predictive biomarker, the findings of the plasma-based analyses represent some of the most promising results to date from the bevacizumab biomarker programme. Furthermore, the findings are supported by recently reported biomarker results from the AVEREL trial of bevacizumab in HER2-positive mBC (Gianni *et al*, 2012). Despite the small sample size in the AVEREL biomarker substudy, the data suggest that high baseline plasma VEGF-A and VEGFR-2 concentrations were associated with greater PFS benefit from bevacizumab, consistent with the AVADO results reported here. A potential predictive role of plasma VEGFR-2 and possibly VEGF-A was also reported recently in the BEATRICE trial in early breast cancer (Carmeliet *et al*, 2012). Accumulating data from several trials support further evaluation of these candidate biomarkers and the potential predictive value of plasma VEGF-A is being evaluated prospectively in trials in other tumour types. Importantly, a prospective trial (MERiDIAN, GO25632) in HER2-negative mBC has started recruitment. Patients are stratified according to baseline plasma VEGF-A concentrations before randomisation to weekly paclitaxel in combination with either bevacizumab or placebo. Until the potential predictive role of plasma VEGF-A has been validated, plasma VEGF-A concentrations cannot be used to guide clinical decision making. Therefore, prospective evaluation of the most promising candidates must be a high priority to enable improved patient selection for the use of bevacizumab in mBC.

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CONFLICT OF INTEREST

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