Published in final edited form as:

Clin Cancer Res. 2011 January 15; 17(2): 372–381. doi:10.1158/1078-0432.CCR-10-1791.

Impact of Exploratory Biomarkers on the Treatment Effect of Bevacizumab in Metastatic Breast Cancer

Adrian M. Jubb¹, Kathy D. Miller², Hope S. Rugo³, Adrian L. Harris⁴, Dafeng Chen⁵, James D. Reimann⁵, Melody A. Cobleigh⁶, Maike Schmidt⁷, Virginia K. Langmuir⁸, Kenneth J. Hillan⁹, Daniel S. Chen⁸, and Hartmut Koeppen⁹

¹Nuffield Department of Clinical Laboratory Sciences, University of Oxford, Oxford, UK.

²University of Indiana Melvin and Bren Simon Cancer Center, Indianapolis, IN.

³Comprehensive Cancer Center, University of California at San Francisco, San Francisco, CA.

⁴Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK.

⁵Department of Biostatistics, Genentech Inc., South San Francisco, CA.

⁶Section of Medical Oncology, Department of Medicine, Rush University Medical Center, Chicago, IL.

⁷Department of Oncology Diagnostics, Genentech Inc., South San Francisco, CA.

⁸Department of Oncology Clinical Development, Genentech Inc., South San Francisco, CA.

⁹Department of Pathology, Genentech Inc., South San Francisco, CA.

Abstract

Purpose—The addition of bevacizumab to cytotoxic chemotherapy has demonstrated a progression free survival (PFS) benefit in the first line and second line treatment of advanced or metastatic breast cancer (MBC). However, the addition of bevacizumab to capecitabine in heavily pretreated MBC patients did not show a PFS benefit (AVF2119g phase three trial). The aim of this study was to evaluate the expression of novel putative biomarkers as predictors of benefit from bevacizumab in retrospective subset analyses of the AVF2119g trial.

Experimental Design—In the AVF2119g trial, 462 patients with MBC were randomly assigned to receive capecitabine or capecitabine plus bevacizumab. Primary tumor tissue and outcome data were available for 223 patients. Biomarker expression was assessed by in situ hybridization (VEGF-A, VEGF-B, thrombospondin-2 and Flt4) or immunohistochemistry (VEGF-C, PDGF-C, neuropilin-1, delta like ligand (Dll)4, Bv8, p53 and thymidine phosphorylase) on formalin fixed paraffin embedded tissue. PFS was associated with these variables in retrospective subset analyses.

Results—Patients with low scores for Dll4, VEGF-C and neuropilin-1 showed trends toward improvement in PFS associated with the addition of bevacizumab to capecitabine (p values 0.01, 0.05 and 0.07, respectively). These observations were not statistically significant following correction for multiple hypothesis testing.

Conclusion—These retrospective subset analyses suggest that expression of Dll4, VEGF-C and neuropilin-1 may predict benefit from bevacizumab. Such observations are not conclusive but warrant additional testing.

Introduction

Angiogenesis is necessary for the growth of tumors,(1) and is predominantly mediated by pro-angiogenic factors (for example, vascular endothelial growth factor-A [VEGF](2)). The expression of VEGF in cancer is controlled by both oncogenic signaling (for instance, epidermal growth factor receptor signaling)(3) and hypoxia.(4) While there is redundancy amongst pro-angiogenic factors in advanced cancer,(5) many early cancers(6, 7) and in vivo models(8) are VEGF dependent. This observation led to the evaluation of an anti-VEGF monoclonal antibody (bevacizumab) with first line cytotoxic chemotherapy in advanced/metastatic breast cancer (MBC), which prolonged the median progression free survival (PFS) in three phase three randomized controlled trials (E2100,(9) RIBBON1, and AVADO).

Two randomized controlled trials have evaluated bevacizumab in patients progressing after first line treatment for MBC. The addition of bevacizumab to second line chemotherapies improved the PFS of patients with MBC by a median 2.1 months (hazard ratio 0.78, $p=0.0072$) in the RIBBON2 trial. However, the earlier AVF2119g trial, evaluating the addition of bevacizumab to capecitabine in first to fifth line MBC (including patients relapsing on therapy), demonstrated a 10.7% improvement in response rate ($p=0.001$) and no PFS benefit.(10) Together with research conducted in preclinical models,(8) the AVF2119g data suggest the possibility that anti-VEGF therapies may be more effective against relatively less advanced tumors that are VEGF dependent. By contrast, relatively more advanced tumors may exploit additional mechanisms to further drive angiogenesis (e.g. platelet derived growth factors [PDGFs])(5, 8) and to establish mature vascular beds that are potentially less sensitive to anti-VEGF therapies.

Preclinical research has provided several new avenues to investigate the impact of bevacizumab on patient survival. Therapies that target VEGF specifically have been observed to selectively prune endothelial cells that are not covered by pericytes.(11) Further research suggests that paracrine endothelial:pericyte signaling, mediated by members of the PDGF family, may account for the relative resistance of more mature vessels to anti-VEGF therapies.(12) Indeed, combined targeting of VEGF and PDGF signaling has additive anti-angiogenic effects.(13) Using a model of resistance to anti-VEGF therapy, Crawford and colleagues reported that stromal expression of PDGF-C is a significant feature of anti-VEGF resistance.(14) Furthermore, di Tomaso and colleagues revealed that overexpression of PDGF-C by glioblastoma xenografts is associated with relative insensitivity to anti-VEGF therapy.(15) No one has tested the implications of these observations in human cancer.

Investigating inherent resistance to anti-VEGF therapy in xenografts, Shojaei and colleagues reported that recruitment of mouse CD11b⁺Gr⁺ myeloid cells was sufficient to confer relative insensitivity to anti-VEGF treatments.(16) Further studies in mouse models suggest that mobilization of CD11b⁺Gr⁺ myeloid cells may be regulated by Bv8 (Bombina variagata peptide 8) expression.(17) Nevertheless, there is no clinical evidence to suggest that these factors are associated with the efficacy of bevacizumab.

Delta-like ligand 4 (Dll4) is a Notch ligand that is expressed by endothelial cells(18) and may be induced by VEGF.(19) Disruption of Dll4 signaling may impair tumor angiogenesis in xenografts.(19, 20) Certain xenografts that are refractory to anti-VEGF therapy are reported to be sensitive to anti-Dll4(19-21) therapy, and combination treatment with anti-

VEGF and anti-Dll4 therapies has additive effects on tumor growth.(19) In addition, human umbilical vein endothelial cells transfected with Dll4 downregulate VEGF receptors and show reduced proliferative and migratory responses to VEGF.(22) Together these data suggest that Dll4 may have a role in mediating resistance to anti-VEGF therapies.

The aim of this study was to evaluate the expression of VEGF pathway components (VEGF-A, VEGF-B, VEGF-C, fms-like tyrosine kinase [flt]4, and neuropilin-1), thrombospondin-2 (an anti-angiogenic gene),(23) p53, thymidine phosphorylase (a putative biomarker of capecitabine efficacy),(24) PDGF-C, Dll4, and Bv8 in available primary cancer tissue as predictors of benefit from bevacizumab in MBC. These hypotheses were tested in exploratory, retrospective subset analyses on the AVF2119g trial of capecitabine with and without bevacizumab in previously treated MBC.

Materials and Methods

Patients and Study Design

Patient recruitment and trial design have been described in detail elsewhere.²¹ In brief, patients with MBC were randomized to receive capecitabine (n=230) or capecitabine with bevacizumab (n=232). Enrollment took place in the United States only from November 2000 to March 2002. Local institutional review boards approved the protocol. At the time of enrollment, informed consent was obtained from trial participants to permit research on their archived tissue. All analyses were performed according to the REMARK criteria.(25)

Tissue Samples

Formalin fixed paraffin embedded tissue blocks and corresponding pathology reports were retrospectively obtained for 181 patients from multiple centers. Where blocks could not be obtained, whole sections were available for 43 patients; the total case series comprised 224 primary tumors. Tissue microarrays were assembled as described previously.(26) Sample size was constrained by the number of tissues available from the pathology archives where the original patient samples were stored.

In Situ Hybridization

Riboprobe synthesis, hybridization, developing, and analysis were carried out as described previously.(27) Sense and anti-sense primers are detailed in Supplementary Table 1.

Hybridization of anti-sense β -actin riboprobes was confirmed in all tissues. Sense riboprobes were employed as negative controls for hybridization specificity. Tissue microarray cores were scored semi-quantitatively on a scale of zero (no expression) to three (very strong signal), according to the overall intensity of the hybridization signal in 10% of neoplastic cells (VEGF-A and VEGF-B), stroma (thrombospondin-2), or endothelium (flt4). The highest score among replicate tissue microarray cores was chosen as the score for the patient. Whole sections were scored on an identical scale. Appropriate tissue and/or cell pellet controls were included in each experiment.(23, 28, 29) Scoring was performed blind to treatment and outcome.

Immunohistochemistry

Immunohistochemistry was performed on freshly cut tissue sections as described previously. The antigen retrieval, primary antibodies, and detection methods used are described in Supplementary Table 2. All antibodies have been previously validated (Supplementary Table 2) except anti-Dll4 and anti-neuropilin-1. The specificity of anti-Dll4 and anti-neuropilin-1 was assessed using human embryonic kidney (HEK)-293 cell pellet controls

transfected with full length human Dll4 and full length mouse Dll4, or full length human neuropilin-1 and empty vector, respectively.

To assess expression, tissue microarray cores and whole sections were scored semi-quantitatively on a scale of zero (no expression) to three (very strong signal), according to the intensity of chromogen deposition in 10% of neoplastic cells (VEGF-C, PDGF-C, neuropilin-1, p53, and thymidine phosphorylase), endothelium (Dll4 and neuropilin-1), or inflammatory cells (Bv8). Whole sections were scored on an identical scale. The highest score among replicate tissue microarray cores was chosen as the score for the patient. Appropriate tissue and/or cell pellet controls were included in each experiment (Supplementary Figures 1 and 2). Substitution of the primary antibody with an isotype control antibody confirmed specificity of immunoreactivity. Scoring was performed blind to treatment and outcome.

Statistical Analyses

To compensate for the large number of statistical hypotheses being tested, a stronger level of evidence is required to determine whether an individual hypothesis is significant. The false discovery rate (FDR) controlling procedure by Benjamini and Hochberg(30) was used to adjust the nominal p values. After adjustment, p values <0.05 were considered significant. Cutoffs were chosen to discriminate between wholly negative cases (score=0) and the remainder, or weakly and strongly positive (cutoff score >1) depending on subgroup size (to ensure a balanced distribution between high and low groups), so that the statistical power can be maximized. Different cutoffs were also used as a sensitivity analysis. Associations between biomarkers were assessed using Pearson's χ^2 test after adjustment for the FDR. Associations between biomarkers and PFS were assessed using survival analyses. All survival analyses refer to PFS from the time of randomization to death or disease progression, whichever comes first (defined by an independent review facility [IRF], investigator's assessment was used for three patients who did not have post-baseline IRF assessments). Patients without an event were censored at the time of the last tumor assessment. Median survival times were estimated from Kaplan-Meier curves, and their 95% confidence intervals were based on the sign test.(31) Hazard ratios and 95% confidence intervals were determined from a Cox regression model with terms of the treatment group, the marker (dichotomized to high and low group), the interaction between the treatment and the marker, and the stratification factors (ECOG performance status (dichotomized to 0 and 1), prior metastatic disease chemotherapy (yes or no)). P values for the hazard ratios were constructed based on Wald tests and then adjusted for the FDR. Sensitivity analyses were performed from a Cox regression model leaving out the stratification factors terms, and a Cox regression model stratified by the stratification factors.

Results

Study Group Characteristics

Outcome data were available for 223 of the 224 patients assessed for biomarker expression (99.6%). The cohort of patients in the biomarker population had demographic and pathological characteristics that were very similar to those in the overall trial population (Table 1). In addition, the estimated PFS treatment effect was not significantly different in the subset of 223 patients compared to the entire study population; the hazard ratio was 0.90 (95% confidence intervals, 0.65 to 1.24) in the subset compared to 0.98 (0.77 to 1.25) in the overall study population. Analyses of tumors from 223 patients yielded informative data on 102 to 183 patients (Table 2). Results were not available for the remaining cases due to limited amounts of tissue.

Biomarker Expression

Expression of tumor VEGF-A (146/183, 80%) and stromal thrombospondin-2 (114/157, 73%) were consistent with detailed descriptions elsewhere(23) (Supplementary Figure 3). Hybridization of the antisense riboprobe for VEGF-B was observed over the tumor cells of 42/102 (41%) cases (Supplementary Figure 3). Hybridization of the antisense riboprobe for Flt4 was observed over endothelial cells in 27/103 (26%) of tumors (Supplementary Figure 3).

Cytoplasmic immunoreactivity for VEGF-C (88/161, 55%) and PDGF-C (90/157, 57%) was predominantly observed in tumor cells (Figure 1). Membranous and/or cytoplasmic neuropilin-1 was observed in tumor associated endothelial cells from 144/162 (89%) patient samples and weakly in the tumor cells of 15/162 (9%) samples (Figure 1 and Supplementary Figure 2). Similarly, membranous and/or cytoplasmic expression of Dll4 was observed in tumor associated endothelial cells from 127/160 (79%) cancers and in a variable proportion of tumor cells in whole sections from approximately 5% of samples (Figure 2 and Supplementary Figure 2). Bv8 (25/161, 15%) was specifically expressed in the cytoplasm of infiltrating inflammatory cells, consistent with previous reports (Figure 2).(32) Nuclear expression of p53(33) (82/174, 47%) and expression of thymidine phosphorylase(24) (127/174, 73%) by tumor cells were consistent with detailed descriptions elsewhere.

Frequency data for in situ hybridization and immunohistochemistry scoring, including cutoffs, are detailed in Table 2. There were no statistically significant differences in frequency data for tissue microarrays (total n=181) and whole sections (total n=43). After correction for multiple testing, only positive associations between thrombospondin-2 and Flt4, PDGF-C and VEGF-C, endothelial Dll4 and endothelial neuropilin-1, and endothelial Dll4 and Bv8 were statistically significant (Supplementary Table 3).

Biomarker Association With Progression Free Survival

Of the biomarkers, only VEGF-A expression by tumor cells showed prognostic significance (though not after FDR correction), a finding previously reported for other tumor types (Supplementary Table 4).(34) VEGF-A, VEGF-B, Flt4, thrombospondin-2, PDGF-C, Bv8 and p53 showed no association with treatment outcome (Table 3). However, subgroups with low expression of endothelial neuropilin-1, thymidine phosphorylase, VEGF-C, or endothelial Dll4 showed trends toward benefit from the addition of bevacizumab to capecitabine in terms of PFS, but after correcting for multiple hypothesis testing, none of them were significant (Table 3 and Figure 3). When endothelial Dll4 subgroups were defined using a cutoff score of 0 or 1 versus 2 or 3 (as opposed to 0 versus 1, 2, or 3), Dll4 did not show statistical significance (data not shown). Expression of neuropilin-1 by tumor cells was not a significant predictive factor (data not shown). Expression of Dll4 by tumor cells was too infrequent to permit meaningful PFS analyses in this series.

Discussion

Treatment regimens that include bevacizumab have demonstrated broad clinical activity.(9, 35-39) However, identification of patient subsets that receive the most clinical benefit would enable more specific treatment administration of bevacizumab and allow patients unlikely to benefit the opportunity to seek other treatment modalities. Unfortunately, despite efforts to identify patient subsets with a differential benefit from bevacizumab, no validated biomarkers have been defined. Research in this area may be complicated by the multiple putative mechanisms of action for bevacizumab and competing biomarkers for sensitivity to co-administered therapy. This study interrogated primary breast cancer tissue collected from a phase III trial of capecitabine with and without bevacizumab, for expression of biomarkers

associated with angiogenesis. None of the biomarkers showed a definitive association with improved PFS. Nevertheless, several trends were identified for expression of VEGF-C and thymidine phosphorylase, neuropilin-1 and Dll4.

Retrospective subset analyses on randomized controlled trials have thus far failed to identify patients with a clinically significant differential benefit from bevacizumab. Published data on colorectal cancer have found no effect of p53 mutations,(40) K-ras/B-raf mutations,(40) VEGF-A expression,(23) microvascular density,(23) thrombospondin-2 expression,(23) or circulating VEGF-A levels(41) on the treatment effect of bevacizumab. Exploratory studies of specific VEGF polymorphisms have been reported to predict overall survival in patients treated with bevacizumab and paclitaxel in MBC in one study,(42) but have not been observed in other large randomized studies of bevacizumab-based treatment. High levels of circulating ICAM (inter-cellular adhesion molecule) were associated with a prolonged overall survival benefit from the addition of bevacizumab to first line chemotherapy in non-squamous non-small cell lung cancer.(43) However, the low ICAM group also derived a small benefit in response rate and overall survival from the addition of bevacizumab, suggesting that ICAM levels have prognostic value and should not be used to select patients.(43) Additional studies have shown changes in angiogenesis related biomarkers in patients who have received bevacizumab, which are suggestive of activity.(44, 45) The authors have yet to relate these observations to survival, making it difficult to evaluate their significance. Circulating endothelial cells (CECs) have been proposed as biomarkers of bevacizumab efficacy.(46) However, to date there are no data that distinguish the predictive efficacy of CECs as regards bevacizumab, from their more general prognostic value(47) in patients not treated with bevacizumab. Preliminary data from Formica and colleagues suggest that circulating CA19.9 may serve as a valid biomarker of survival benefit, though this will need to be confirmed in a trial population.(48)

This study aimed to evaluate several putative biomarkers of anti-VEGF efficacy identified by preclinical research. There is growing evidence from model systems that Dll4 expression may define tumors that are resistant to anti-VEGF therapies and inhibition of Dll4 may overcome resistance to bevacizumab.(21, 22) Herein, tumors with low endothelial Dll4 expression showed a trend toward a benefit in PFS from the addition of bevacizumab to capecitabine (median 4.53 month improvement, $p=0.01$). No benefit was observed in tumors with high endothelial Dll4 expression. In addition, patients from the low Dll4 subset who were treated with capecitabine showed a marginally worse PFS than patients from the high Dll4 subset, though this was not statistically significant. The hypothesis follows that tumors with high endothelial Dll4 expression have more mature vasculature, but tumors with low endothelial Dll4 expression have immature, poorly functional vasculature, leading to hypoxia, which is an adverse prognostic factor. When bevacizumab is combined with capecitabine, there is an anti-angiogenic effect with normalization of the vascular bed in tumors with immature vessels expressing low levels of Dll4. The effect of bevacizumab is, perhaps, more restricted in tumors with mature, functional vasculature expressing high levels of Dll4. However, the number of patients and events in these subgroups is very small, which leaves these analyses open to bias from imbalances in patient characteristics. While there is a biological rationale to support these observations, the data are exploratory and independent validation should be sought.

Several other biomarkers showed trends toward benefit from bevacizumab, including low expression of VEGF-C, endothelial neuropilin-1, and thymidine phosphorylase. Redundancy among VEGF ligands may provide a mechanism for tumor escape from anti-VEGF agents. VEGF-C and its receptor Flt4 have a physiological role in lymphangiogenesis,(49) but a pathological role in angiogenesis of breast cancer.(50) Expression of VEGF-C by tumor cells and Flt4 by tumor-associated endothelium may invoke resistance to anti-VEGF-A

therapies by providing an alternative pathway for angiogenesis. In addition, VEGF-C is believed to be capable of signaling through VEGF receptor 2.(51) Neuropilin-1 is a cell surface protein that can directly interact with VEGF-A and VEGF receptor 2, and may be expressed by both tumor and endothelial cells.(52) Data from Pan et al. suggest that combined inhibition of neuropilin-1 and VEGF-A has additive anti-angiogenic effects, prohibiting the organization of endothelium and pericytes to form mature vessels.(52) Therefore, it is possible that endothelial neuropilin-1 expression may identify a group of patients amenable to combination anti-neuropilin-1 and anti-VEGF treatment.

High expression of thymidine phosphorylase, an enzyme involved with the metabolism of capecitabine to 5-fluorouracil, has been reported to predict benefit from capecitabine.(24) Herein, the median PFS in the capecitabine treatment arm was 1.97 months longer in the subgroup with high versus low thymidine phosphorylase expression. Median PFS was similar for low and high expressing thymidine phosphorylase subgroups in the capecitabine plus bevacizumab treatment arm. Hence the perceived benefit from bevacizumab in tumors expressing low levels of thymidine phosphorylase may be an artifactual reflection of the prognostic effect of high thymidine phosphorylase expression in the control arm.

Due to the limited availability of tissue from metastases, these analyses have used primary tumor samples to predict the behavior of metastatic disease. One may expect differences between primary cancers and their metastases, especially in terms of tumor-stromal interactions and angiogenesis, depending on the host tissue. Further clinical research is needed to investigate the impact of these differences on the observations made herein and elsewhere in biomarker studies.

The lack of an observed predictive effect of VEGF-A and p53 expression is consistent with published predictive studies on the survival benefit of bevacizumab.(23, 40, 42) Despite preclinical evidence to suggest a predictive effect,(14, 17) neither Bv8 nor PDGF-C expression were significant biomarkers of benefit from the addition of bevacizumab to capecitabine. However, this may be related to dynamic regulation of tumor expression of these factors. Given the potentially long time period between primary breast tumor collection and second or third line treatment with chemotherapy for metastatic breast cancer on this study, it is conceivable that tumor expression of these factors had changed substantially. In addition, it is noted that very few of these archival primary breast tumor samples tested expressed Bv8 by immunohistochemistry, preventing an adequate comparison of treatment outcome for patients with high versus low Bv8 expression.

These exploratory analyses suggest that several biomarker subsets show trends toward improved PFS from the combination of bevacizumab with capecitabine in patients with previously treated MBC. However, the observations are not statistically significant after correction for multiple hypothesis testing. Validation and more fundamental research on the appropriate clinical material are required to understand the complexity of angiogenesis and response to antiangiogenic therapy.

Statement of Translational Relevance

The clinical benefit of bevacizumab in metastatic breast cancer is small and an overall survival benefit is not seen. Furthermore, there are no validated biomarkers of the survival benefit afforded by bevacizumab. Objective response is not predictive of the survival benefit afforded by bevacizumab in colorectal cancer, and improved objective response rates are seen in the absence of a survival benefit in breast cancer. Oncologists cannot objectively select patients to start bevacizumab and cannot tell when resistance develops. This analysis assesses biomarkers that have been shown to be functionally involved in resistance to bevacizumab in preclinical studies. The predictive impact of

these markers has not been assessed before in terms of survival data from a randomized controlled trial. Our data suggest that these markers may have predictive significance and is a first step in identifying patients who benefit from bevacizumab and resistant patients who may benefit from alternative therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank all patients and investigators who participated in the AVF2119g clinical trial. Members of Genentech's immunohistochemical, in situ hybridization and core pathology laboratories made invaluable contributions to the implementation of this study.

Financial Support: This work was funded by Genentech Inc. Dr Adrian M. Jubb is supported by a Career Development Fellowship from the Pathological Society of Great Britain and Ireland. Professor Adrian L. Harris receives funding from Cancer Research UK, the Oxford Biomedical Research Centre and the European Union METOXIA Research Framework.

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Figure 1.

Representative immunohistochemistry for vascular endothelial growth factor-C (A, tumor 2+), platelet derived growth factor-C (B, tumor 2+) and neuropilin-1 (C, endothelium 2+; D, endothelium 2+, tumor +). Brown staining with diaminobenzidine indicates immunoreactivity.

Figure 2.

Representative immunohistochemistry for delta-like ligand 4 (A, endothelium score 2; B, endothelium 3+, tumor +), Bombina variagata peptide 8 (C, inflammation 3+), and thymidine phosphorylase (D, tumor 1+). Brown staining with diaminobenzidine indicates immunoreactivity.

Figure 3.

Kaplan-Meier progression-free survival curves of patient groups treated with capecitabine or capecitabine plus bevacizumab. Subsets of cancers with low (A) and high (B) endothelial Dll4 expression are shown. Censored patients are indicated by a circle (capecitabine plus bevacizumab) or a cross (capecitabine). Dll4, delta-like ligand 4.

Table 1

Patient characteristics.

Characteristic	Parent Study		Sub-study	
	Capecitabine (230 patients)	Capecitabine plus Bevacizumab (232 patients)	Capecitabine (101 patients)	Capecitabine plus Bevacizumab (122 patients)
Age, years				
Mean	52	51	51	50
Range	30-77	29-78	30-76	32-75
Race				
Black	25 (10.9%)	30 (12.9%)	11 (10.9%)	21 (17.2%)
White	185 (80.4%)	187 (80.6%)	77 (76.2%)	95 (77.9%)
Other	20 (8.7%)	15 (6.5%)	13 (12.9%)	6 (4.9%)
ECOG PS				
0	115 (50.0%)	117 (50.4%)	51 (50.5%)	67 (54.9%)
1	115 (50.0%)	114 (49.1%)	50 (49.5%)	55 (45.1%)
2	0	1 (0.4%)	0	0
ER positive	119 (51.7%)	97 (41.8%)	53 (52.5%)	47 (38.5%)
PR positive	96 (41.7%)	75 (32.3%)	41 (40.6%)	42 (34.4%)
HER2 positive	47 (20.4%)	61 (26.3%)	21 (20.8%)	41 (33.6%)
Duration of metastatic disease, years				
Median	1.3	1.0	1.0	0.8
Range	0-19.3	0-13.2	0-19.3	0-13.2
Visceral disease	184 (80%)	180 (77.6%)	79 (78.2%)	93 (76.2%)
3 Sites of disease	116 (50.4%)	114 (49.1%)	47 (46.5%)	59 (48.3%)
Prior chemotherapy regimens for MBC				
0	37 (16.1%)	35 (15.1%)	20 (19.8%)	23 (18.9%)
1	98 (42.6%)	107 (46.1%)	43 (42.6%)	55 (45.1%)
2	87 (37.8%)	79 (34.1%)	34 (33.7%)	40 (32.8%)
3-5	8 (3.5%)	11 (4.7%)	4 (4.0%)	4 (3.3%)
Progression-Free Survival Benefit from the Addition of Bevacizumab to Capecitabine (Hazard Ratio with 95% Confidence Intervals)	0.90 (0.65 to 1.24)		0.98 (0.77 to 1.25)	

Abbreviations: PS, performance status; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; MBC, metastatic breast cancer.

Table 2

Frequency Data for In Situ Hybridization and Immunohistochemical Scoring

Marker	Cutoff Low/High	Score 0		Score 1		Score 2		Score 3		Total
		No.	%	No.	%	No.	%	No.	%	
VEGF-A	0,1 vs 2,3	37	20	79	43	38	21	29	16	183
VEGF-B	0 vs 1,2	60	59	41	40	1	1	0	0	102
Flt4	0 vs 1,2	76	74	24	23	3	3	0	0	103
TSP2	0,1 vs 2,3	43	27	55	35	47	30	12	8	157
VEGF-C	0 vs 1-3	73	45	36	22	35	22	17	11	161
PDGF-C	0 vs 1,2	67	43	71	45	19	12	0	0	157
Neuropilin-1	0,1 vs 2,3	18	11	78	48	65	40	1	1	162
Dll4	0 vs 1-3	33	21	90	56	35	22	2	1	160
Bv8	0 vs 1-3	136	85	15	9	5	3	5	3	161
p53	0 vs 1-3	92	53	44	25	10	6	28	16	174
TP	0 vs 1	47	27	127	73	0	0	0	0	174

Abbreviations: VEGF, vascular endothelial growth factor; flt4, fms-like tyrosine kinase 4; TSP2, thrombospondin 2; Bv8, Bombina variagata peptide 8; PDGF-C, platelet-derived growth factor C; Dll4, delta-like ligand 4; TP, thymidine phosphorylase; vs, versus.

Table 3

Cox-Regression Analysis of Time To Progression-Free Survival for Each Biomarker Subgroup, After Adjustment for Stratification Factors and Interaction Between Treatment and the Biomarker Variable.

Marker	Total Patients (n)	Capecitabine				Capecitabine plus Bevacicunab				Hazard Ratio	95% CI of the Hazard Ratio	p value Wald / FDR
		Patients (n)	Events (n)	Median Time to Event (months)	95% CI of the Median Time to Event	Patients (n)	Events (n)	Median Time to Event (months)	95% CI of the Median Time to Event			
Total	223	101	67	3.78	2.89-4.86	122	84	4.60	4.01-5.45	0.90	0.65-1.24	0.51
VEGF-A												
High	67	23	18	2.89	2.56-4.11	44	29	4.01	2.79-5.42	0.72	0.39-1.32	0.29 / 0.71
Low	115	54	33	4.11	2.89-9.07	61	41	5.45	4.30-7.72	0.89	0.56-1.41	0.60 / 0.94
VEGF-B												
High	42	20	13	4.86	2.89-9.07	22	14	5.42	4.60-7.72	1.14	0.52-2.51	0.74 / 0.86
Low	59	25	14	3.78	2.83-12.58	34	24	4.30	2.79-6.77	1.14	0.58-2.22	0.71 / 0.86
Flt4												
High	27	13	6	5.75	2.89-9.07	14	10	4.01	2.14-7.72	1.35	0.48-3.76	0.57 / 0.86
Low	75	33	22	3.65	2.83-9.23	42	28	5.36	4.24-6.57	1.04	0.2358-1.86	0.89 / 0.89
TSP2												
High	59	26	17	3.09	2.83-4.40	33	20	5.39	4.01-6.77	0.83	0.43-1.60	0.58 / 0.86
Low	97	42	25	5.78	3.52-9.23	55	42	4.76	3.94-5.49	1.19	0.72-1.97	0.50 / 0.86
VEGF-C												
High	87	39	24	4.27	2.79-9.46	48	30	4.86	3.78-5.78	1.11	0.64-1.91	0.71 / 0.86
Low	73	32	26	2.89	1.51-4.14	41	32	4.60	2.79-5.52	0.59	0.35-1.01	0.05 / 0.39
PDGF-C												
High	89	39	25	4.40	2.89-9.07	50	36	4.86	2.83-5.52	1.10	0.66-1.85	0.72 / 0.86
Low	67	30	23	2.89	1.77-4.14	37	27	4.17	2.79-5.49	0.70	0.40-1.23	0.21 / 0.70
Neuropilin-1												
High	66	34	25	3.02	2.76-5.62	32	27	4.17	3.55-5.45	1.34	0.75-2.40	0.32 / 0.70

Marker	Total Patients (n)	Capecitabine			Capecitabine plus Bevacicunab					Hazard Ratio	95% CI of the Hazard Ratio	p value Wald / FDR
		Patients (n)	Events (n)	Median Time to Event (months)	95% CI of the Median Time to Event	Patients (n)	Events (n)	Median Time to Event (months)	95% CI of the Median Time to Event			
Low	95	37	25	3.52	2.69-5.13	58	36	5.39	3.48-7.72	0.61	0.36-1.03	0.07 / 0.3 9
DI14												
High	126	59	41	4.11	2.83-5.49	67	51	4.17	3.55-5.45	1.05	0.69-1.60	0.82 / 0.86
Low	33	12	9	2.17	1.41-3.02	21	11	6.70	2.83-8.38	0.31	0.13-0.77	0.01 / 0.2 2
Bv8												
High	25	13	8	4.86	2.89-12.58	12	10	2.43	1.38-4.14	1.78	0.65-4.86	0.26 / 0.70
Low	135	58	42	3.02	2.56-4.27	77	53	4.86	4.01-5.52	0.77	0.51-1.15	0.20 / 0.70
p53												
High	81	34	23	3.78	2.89-4.86	47	34	4.30	3.48-5.45	0.92	0.54-1.56	0.74 / 0.86
Low	92	43	24	5.62	3.65-9.07	49	32	5.36	4.01-5.78	1.07	0.62-1.85	0.80 / 0.86
TP												
High	127	54	32	4.86	3.71-8.57	73	52	4.60	4.01-5.45	1.30	0.83-2.03	0.25 / 0.70
Low	46	24	16	2.89	2.30-5.45	22	14	4.86	3.94-7.75	0.50	0.24-1.06	0.07 / 0.3 9

Abbreviations: FDR, false discovery rate; VEGF, vascular endothelial growth factor; flt4, fms-like tyrosine kinase 4; TSP2, thrombospondin 2; DI14, delta-like ligand 4; Bv8, Bombina variagata peptide 8; PDGF-C, platelet-derived growth factor C; TP, thymidine phosphorylase; CI, confidence intervals.