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High Circulating Tie-2 is Associated with Pathologic Complete Response to Chemotherapy and Antiangiogenic Therapy in Breast Cancer

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Abstract

Vascular endothelial growth factor (VEGF) is a central mediator of angiogenesis in breast cancer. Research in antiangiogenic cancer treatment has been marked by the development of the monoclonal antibody bevacizumab, which targets VEGF in many solid tumors. Since patients do not equally benefit from bevacizumab, it has become necessary to define the profile of patients who will benefit from the drug.

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Patients and methods—We have conducted a prospective phase II study in 39 patients using bevacizumab in breast cancer in the neoadjuvant setting, and found improved pathologic complete response (pCR) when bevacizumab was added to chemotherapy in patients with hormone receptor negative and invasive ductal carcinoma. Blood samples were collected at baseline and serially while patients were on treatment. Circulating angiogenesis-related proteins ANG1, ANG2, bFGF, IL-1a, MMP-9, PDGF-BB, PECAM-1, Tie-2, VEGF, and VEGFR2 were measured at baseline and during treatment. This correlative study was conducted to identify specific serum angiogenic factor profiles that might be associated with pCR in the neoadjuvant setting in breast cancer patients receiving bevacizumab and chemotherapy.

Results—Elevated baseline serum Tie-2 and bFGF were associated with pCR in response to this combination. Changes in serum levels of these proteins were seen during treatment but were not significantly different between the pCR and non-pCR groups.

Conclusion—Baseline circulating Tie-2 levels may help distinguish patients who will have pCR from those who will not and may form the basis for future development of antiangiogenic therapy in breast cancer. Larger studies are needed to validate these findings.

Keywords

Breast cancer; neoadjuvant chemotherapy; antiangiogenic therapy; pathologic complete response; Tie2

Introduction

VEGF is a central mediator of angiogenesis and the first proangiogenic factor released by breast cancer.¹⁻⁶ It remains the main pro-angiogenic factor until late in the progression of this cancer, which suggests more benefit from bevacizumab therapy in early stage breast cancer.^{7,8} Bevacizumab is a monoclonal antibody that binds all isoforms of VEGF and leads to the removal of these proteins from the tumor microenvironment. Animal studies using xenograft models showed that tumors regress when this pathway is targeted.⁹ However, results in humans have not been as striking. As a single-agent, bevacizumab showed minimal-to-no efficacy, but the combination of bevacizumab with paclitaxel led to a doubling of the response rate (18% vs. 36%) and PFS (5.8 mo. vs. 11.6 mo.) in metastatic breast cancer.¹⁰ Unfortunately, the treatment carries the risk of arterial thromboembolic complications, GI perforation, heart failure and bleeding; therefore it is extremely important to offer this treatment only to the patients who are more likely to benefit from it.¹¹⁻¹⁴

Physiologic and tumor angiogenesis are marked by redundancy.¹⁵ Although VEGF is the main proangiogenic factor in both circumstances, it has been shown that many proangiogenic and antiangiogenic factors are at work and their intervention varies depending on the type of the tumor and its stage. Angiopoietins belong to a family of secreted proteins that all bind to an endothelial receptor, Tie2 (tyrosine kinase with immunoglobulin and epidermal growth factor-homology domains 2). Angiopoietin 1 (ANG1) is produced by fibroblasts, non-vascular normal and tumor cells and pericytes. It binds specifically to Tie2, leading to its activation by inducing its phosphorylation. ANG1 does not promote the growth of cultured endothelial cells, but does induce their migration, tube formation, sprouting and

survival, and has anti-permeability and anti-inflammatory properties.¹⁶ In vivo, it maintains quiescence of the vasculature.¹⁷ Angiopoietin2 (ANG2) is mainly produced by endothelial cells, and promotes vessel destabilization. It works as an antagonist of ANG1 by binding to Tie2 without inducing its phosphorylation. It is expressed in conjunction with VEGF at sites of vessel sprouting and growth and alone at sites of frank vessel regression (e.g. atretic follicles). ANG2 is induced by VEGF and other factors such as hypoxia,¹⁷ and promotes the removal of pericytes from the endothelium.¹⁸ Once the primitive vessels are formed and blood starts flowing through the network, hypoxia decreases, leading to downregulation of VEGF, which leads to downregulation of ANG2 and an increase in the ratio of ANG1/ANG2. High ratios of ANG1/ANG2 and high levels of PDGF serve to recruit pericytes and achieve the maturation of blood vessels. Mature blood vessels are less leaky and less dependent for their survival on VEGF.

The current study was begun to unravel a reliable circulating angiogenic factor profile associated with pCR in response to the combination of chemotherapy with the antiangiogenic drug bevacizumab in breast cancer. It is likely that breast cancer is associated with different angiogenic profiles and would respond to different types of antiangiogenic therapy. This heterogeneity stems from (1) the major differences in activated pathways in breast cancer that lead to different intrinsic subtypes¹⁹ with distinct gene expression profiles and (2) from germline differences in engaging angiogenic pathways. We hypothesized that individuals who achieved pCR have a distinct profile of circulating angiogenesis-related proteins with a focus on VEGF-VEGFR, Tie2-ANG1/2 and PDGF-PDGFR pathways that may be predictive of pCR in the neoadjuvant setting.

Materials and Methods

Clinical Approach

The role of VEGF inhibition in the neoadjuvant treatment of breast cancer using bevacizumab and chemotherapy (CT) in an IRB-approved, prospective single-arm, single-institution phase II efficacy trial has previously been reported.²⁰ Briefly, we approached patients with proven stage II or III breast cancer and offered them CT combination with docetaxel (75 mg/m²), cyclophosphamide (500 mg/m²) and bevacizumab (15 mg/kg) every 3 weeks × 4 cycles followed by doxorubicin (60 mg/m²) every 3 weeks × 4 cycles before undergoing surgery for their cancer. After surgery, patients were evaluated for pathologic complete response (pCR), defined as the absence of all evidence of invasive cancer in the breast specimen. Forty patients were enrolled, but one withdrew consent; 38 of the remaining 39 underwent surgery. The pCR rate was 41% (16/39), significantly higher than the null hypothesis rate of 25% ($P=0.020$).

Sample collection, separation and storage

Blood samples were collected from patients at baseline and just before infusion at each of the 8 pre-operative chemotherapy cycles. Blood components were separated, aliquoted into cryovials and stored at -80°C for later analyses.

Protein Analysis Method

To test the hypothesis that individuals who achieved pCR have a different concentration of circulating angiogenesis-related proteins, we sought to determine the serum concentrations of ten proteins, ANG1, ANG2, bFGF, IL-1a, MMP-9, PDGF-BB, PECAM-1, Tie-2, VEGF, and VEGFR2. Coded serum samples were sent for commercial assay of protein concentrations via custom antibody array using the Quantibody® array-based multiplex ELISA system (RayBiotech, Inc., Norcross, GA, USA). Quantification of unknowns was achieved using log-log standard curves constructed from pre-determined serial dilutions of cytokine standards. The assays were performed blinded to the study endpoint.

Study Design

The original clinical study was a single arm, single institution study designed to test the hypothesis that the addition of bevacizumab to chemotherapy would increase pCR by 15% over chemotherapy alone (null hypothesis, pCR of 25%). All 40 patients received the same regimen that included bevacizumab and chemotherapy. The primary clinical end point was pCR that was defined as the absence of invasive cancer in the breast. pCR in the breast and axilla data were collected but the study was not powered for this endpoint. Variables initially examined in the clinical study were age, race, stage, grade, ER/PR and HER2 status and the following angiogenic blood markers were assessed after the completion of study: ANG1, ANG2, bFGF, IL-1a, MMP-9, PDGF-BB, PECAM-1, Tie-2, VEGF, and VEGFR2. The original sample size was determined based on the hypothesis that the percentage of pCR cases with this combination of bevacizumab and chemotherapy was 15% higher than a null hypothesis of 25% with chemotherapy alone with one sided p value of < 0.05 .²⁰

Statistical Analysis

Study subjects were included in the analysis if they had at least two serum samples analyzed, one collected at baseline and one or more collected during pre-operative chemotherapy. For each patient, the maximum value of each protein after baseline was determined along with the difference between that protein's maximum and baseline values. Baseline values, maximum values, and differences from baseline were summarized by protein and pCR status as medians and interquartile ranges, and compared via Wilcoxon rank-sum test for differences between the pCR and non-pCR groups. Logistic regressions adjusting for ER/PR status were used to assess the baseline levels of each protein for its potential to help predict subsequent response to therapy. To make results comparable in the logistic regressions, each protein was normalized by (a) median-centering the baseline concentrations and (b) dividing the result by the interquartile range (IQR) at baseline. This procedure assured for each protein that its unit of increase in the logistic regression was equal to its IQR at baseline. The significance level was set at $\alpha=0.05$ despite the multiple comparisons, in order not to inflate Type II (false negative) error in this modestly powered exploratory correlative study. Estimates of sensitivity and specificity were accompanied by 2-sided 95% confidence limits calculated assuming asymptotic normality.

Results

Patient Characteristics

Of the 39 study subjects, 34 met the serum-sample criterion, including 12 (35%) who had attained pCR. These 34 included 8 (24%) African Americans, 24 (71%) Caucasians, one Hispanic, and one Asian. Their median age was 46.5 years, and their median BMI was 25.9 kg/m². Fourteen (41%) were ER/PR –/–, 27 (79%) were HER2/Neu negative, and 11 (32%) were triple-negative. Twenty five (74%) had ductal carcinoma, 20 (59%) had grade III disease, and 26 (76%) received mastectomy. Table 1 shows the distribution of patient characteristics among subjects who attained pCR compared to subjects who did not. Only hormone-receptor status differed significantly between groups, with 67% (8/12) of pCR subjects being ER/PR –/– compared to only 27% (6/22) of non-pCR subjects ($P=0.026$). Interestingly, ductal carcinomas constituted 92% (11/12) of pCR subjects compared to only 64% (14/22) of non-pCR subjects, but the difference only trended towards significance ($P=0.077$). None of the other patient characteristics showed a statistically significant difference between subjects who attained pCR and subjects who did not.

Serum Proteins at Baseline

Table 2 shows, in picograms/milliliter (pg/mL), the medians, quartiles, and ranges of concentrations of 10 proteins measured in serum samples collected before treatment from subjects who subsequently attained pCR and from subjects who did not. Two proteins, Tie-2 and bFGF, showed significantly higher pre-treatment concentrations in subjects who attained pCR. The median (quartiles) of Tie-2 was 617 (514–870) pg/mL in the pCR group, versus only 416 (303–467) pg/mL in the non-pCR group ($P=0.0013$). In the case of bFGF, concentrations were below the limit of detection at 33.3 pg/mL in only 17% (2/12) of pCR samples, but fully half (11/22) of non-pCR samples. The median (quartiles) of bFGF was accordingly 73.4 (36.7–137.0) pg/mL in the pCR group, versus <33.3 (<33.3–68.7) pg/mL in the non-pCR group ($P=0.045$). The other eight proteins showed no significant pre-treatment difference between subjects who attained pCR and subjects who did not.

Serum Proteins during treatment

Table 3 summarizes the pCR and non-pCR groups: (a) the maximum post-baseline value attained by each serum protein, and (b) the within-patient difference from each protein's maximum to its baseline. Neither the maxima nor their paired differences from baseline were statistically significantly different between subjects who attained pCR and subjects who did not.

Serum Proteins as Predictive Markers of Response to Therapy

As noted above, hormone-receptor status was the only patient characteristic that was significantly associated with responsiveness to bevacizumab and chemotherapy (Table 1). A subsequent logistic-regression analysis disclosed that subjects who were doubly negative for ER and PR were more than 5 times likelier to attain pCR than subjects who had at least one positive receptor (Odds Ratio (OR) = 5.33, 95% Confidence Interval (CI) 1.16–24.5; $P=0.031$), thereby implicating ER/PR status as a marker predictive of response to the

combined chemo-bevacizumab therapy. To determine whether any of the serum proteins could “add value” to the predictive ability of ER/PR status, we added the baseline levels of each serum protein to ER/PR status in the logistic-regression model, and obtained the results shown in Table 4. For each protein, the left half of Table 4 shows the adjusted OR favoring pCR with a one-IQR increase in the protein's baseline concentration, while the right half of Table 4 shows the adjusted OR for ER/PR status in the presence of the serum protein. When Tie-2 was added to ER/PR status, not only was Tie-2 significant (adjusted OR=3.70 with a 1-IQR increase; $P=0.021$), but ER/PR status became non-significant (adjusted OR=3.92 with “–/–”; $P=0.12$), indicating that Tie-2 levels account for some of the predictiveness of ER/PR status. Interestingly, baseline IL-1a levels did not differ significantly between pCR and non-pCR groups (Table 1), and was not significantly predictive of response in univariate logistic regression (crude OR=1.99 with a 1-IQR increase; $P=0.18$). However, baseline IL-1a added to ER/PR status in the logistic regression approached significance (adjusted OR=3.05 with a 1-IQR increase; $P=0.053$) and also potentiated the predictiveness of ER/PR status (adjusted OR=9.32 with “–/–”; $P=0.016$), indicating that IL-1a may have predictive potential when ER/PR status is taken into account.

We also examined Tie-2's predictiveness of response to bevacizumab therapy when it was dichotomized at a suitable cut-point. When Tie-2 was dichotomized at 550 pg/mL and added to ER/PR status in the logistic regression, high Tie-2 was significantly predictive of subsequent pCR (adjusted OR=10.1, 95% CI 1.70–59.7; $P=0.011$), whereas doubly negative ER/PR was not (adjusted OR=3.74, 95% CI 0.66–21.3; $P=0.14$). When ER/PR status was removed from the model, high Tie-2 was significantly predictive of subsequent pCR (crude OR=12.7, 95% CI 2.29–70.0; $P=0.0036$). Tie-2 dichotomized at 550 pg/mL successfully predicted 8 of the 12 subjects who would attain pCR (sensitivity=67%, 95% CI 40%–93%), and successfully predicted 19 of the 22 subjects who would not attain pCR (specificity=86%, 95% CI 72%–100%).

Discussion

The challenges facing the search for markers that are predictive of response to bevacizumab therapy were recently reviewed.²¹ Among the reasons for the failure to identify a “marker” is the multiplicity and redundancy of factors involved in angiogenesis and its dynamic nature. The “true” mechanism of action of bevacizumab remains elusive and it is likely that the impact of VEGF blockade will vary depending on the type of tumor, its size or stage and the host “angiogenic profile”. Furthermore, most studies addressing the correlation between angiogenesis markers and clinical outcomes (PFS and OS) were conducted in the metastatic setting, which makes it difficult to extrapolate the results to the adjuvant or neoadjuvant setting. These studies showed no correlation between objective response and PFS or OS. Lastly, our study tried to address the question of predictors of response to the combination of bevacizumab and chemotherapy as it was a single arm study with no control arm without bevacizumab. It is possible that similar findings can be found for chemotherapy alone but we do not have these data.

One of the issues associated with the use of serum biomarkers to predict response in the tumor is the uncertainty about the correlation between the serum levels and the levels of the

same markers in the microenvironment of the tumor. Thus, serum total VEGF levels are not predictive of tumor response, as they are the reflection of the systemic production of this ubiquitous protein, and the tumor contribution to these levels may not be significant. In order to overcome this difficulty, studies focused on measuring the diffusible forms of VEGF (VEGF-A¹¹⁰⁻¹²¹), a protein that might better reflect tumor angiogenesis. Using a specific ELISA test with a preference for short (VEGF110, VEGF121) over longer (VEGF165, VEGF189) isoforms, Van Cutsem and colleagues demonstrated that high circulating short VEGF-A levels were predictive of improved overall survival in advanced gastric cancer patients treated with bevacizumab in combination with chemotherapy.²² The same study indicated that low levels of circulating neuropilin-1 were also predictive of bevacizumab efficacy.

Our study was conducted in the neoadjuvant setting and assessed pCR in hormone receptor positive and negative patients with large breast tumors. Consistent with the possible role of redundant/parallel systems to either achieve maturation or to maintain survival of tumor blood vessels after the use of bevacizumab, we focused on multiple markers to predict the behavior of different types of breast cancers. In order to define “patient angiogenic profiles”, ten protein markers were measured in the serum at base line and during treatment: ANG1, ANG2, Tie-2, bFGF, IL-1a, MMP-9, PDGF-BB, PECAM-1, VEGF, and VEGFR2. The levels of ANG1/2 or their ratios were not predictive of pCR while the levels of Tie2 were associated with pCR. Baseline bFGF levels were also higher in patients who achieved pCR and this finding was statistically significantly. The biological significance of this finding related to bFGF is still under investigation, since the levels of bFGF were near the limit of detection of the method in many cases.

ANG 1/2 - Tie2 and bFGF are parallel angiogenic systems that interact with VEGF to ensure development of new blood vessels. ANG2 is induced by VEGF, and since ANG1 levels are relatively constant the ratio of ANG1/ANG2 is decreased in areas of increased angiogenesis where VEGF is activated. Our study was designed to identify a serum-based protein profile that may be of predictive/prognostic utility for the medical oncologist without direct examination of the tumor tissue. Importantly, our results do not necessarily correlate with changes in the tumor microenvironment.

The biological origin of circulating Tie2 is not known. The polyclonal antibody used in the serum assay targets an area of the Tie2 receptor spanning from Ala23 to Lys745 and is mostly located in the extracellular domain. Four splice variants of Tie2 were described (1124, 1081, 976 and 468 aa), and two of them had their transmembrane domain falling within the area measured by the antibodies (transmembrane domain location for the largest splice variants: 748-770, 705-727, 601-623). The third splice variant consists exclusively of the extracellular domain.^{21,23} It is conceivable that the extracellular domain of Tie2 may undergo cleavage under the effects of proteases at a site close to AA 600-747. This is likely to happen in areas of tissue remodeling and active angiogenesis such as wound healing and the tumor microenvironment. If the antibody targets an epitope of Tie2 closer to Ala23, it will detect both the intact and cleaved Tie2, and will not be able to discriminate which one is detected. However, since the detected segment spans the transmembrane domain in at least two of the splice variants, it is possible that the protein detected reflects its systemic

production by normal endothelial and bone marrow cells that express this receptor. Alternatively, the circulating Tie2 may reflect its shedding from endothelial cells under the effect of VEGF as it was shown in a limb ischemia model.²⁴ The fact that Tie2 levels did not drop during treatment in patients who achieved pCR suggests that these levels are a host rather than a tumor characteristic.

The interaction between the unique “angiogenic profile” and the tumor type may result in some cases in a critical dependence of tumor vasculature on VEGF (hence high possibility to respond to bevacizumab) or lack of dependence on VEGF (therefore no or little response to bevacizumab). Our previously published clinical data suggested that only hormone receptor negative breast cancers have high probability to respond but not all of them do. The current study revealed that patients with high circulating Tie2 are the most likely to respond. This finding has a biological significance as the Tie2-ANG1/2 system is intimately involved in blood vessel maturation. Whether high circulating Tie2 is the result of its systemic production by normal tissues or the result of its shedding at the tumor site or both, the final result might be the same, i.e. higher dependence of the tumor on VEGF.

High circulating Tie2 levels were found to inhibit and induce regression of corneal neovascularization in BALB/c mouse model.²⁵ High circulating concentrations of Tie2 may lead to high Tie2 concentrations in the microenvironment of the tumor, and this in turn might play the role of a “sink” or decoy to ANG1/2. As free Tie2 binds these two proteins, it prevents them from binding to their endothelial Tie2 receptor. For a given level of production of local ANG1/2, high concentrations of circulating Tie2 would lead to lower availability of these two ligands to exert their local effect. Thus, tumors in patients with high circulating concentrations of Tie2 may be more dependent on an intact VEGF pathway than the ones with low circulating Tie2. The depletion of VEGF from the tumor microenvironment in patients with high circulating Tie2 levels after the administration of bevacizumab may have a more pronounced effect on angiogenesis compared to patients with low circulating Tie-2 levels. These findings are clinically relevant, since drugs are now available that can mimic the effect of circulating Tie2, such as AMG-386, a peptibody that binds ANG1/ANG2.¹⁷

The reason for the discrepancy in the results from previously published clinical trials^{26,27} about the role of bevacizumab in breast cancer is the fact that only a small percentage of patients have tumors that are critically dependent on the VEGF pathway. In our estimate, patients who have a chance to respond to this drug are the triple negative group (10% to 15% of all breast cancer patients) and those with the “highest” chance are those with high Tie2 (around 10% of all breast cancer patients). If this is really the case one might understand why even large clinical trials were not able to show a clear-cut efficacy of this agent in all comers.

The current data, along with our recently reported clinical outcomes from this study, will aid in the design of the next generation studies. From a clinical standpoint, future studies should target patients with triple-negative invasive ductal carcinoma of the breast. One of the limitations of our study is the modest number of patients, which precluded adjustments for multiple testing. Therefore, our results should be considered exploratory, and they require

confirmation. Considering the small size of the study, checking assumptions, sensitivity analyses, and internal validation were not addressed. Hence, our compliance with the REMARK guidelines was partial, which is considered an acceptable practice in “early marker studies.”²⁸ Further pre-clinical and clinical studies appear warranted to more broadly define a protein-biomarker signature in breast cancer patients that would allow personalized treatment regimens to be used in potential responders. Once the predictive role of Tie2 level is confirmed, high risk triple negative, invasive ductal carcinoma breast cancer patients may be offered chemotherapy with bevacizumab if Tie2 levels are high or chemotherapy with bevacizumab and AMG386 if Tie2 is low. Thus, patients who are unlikely to respond to the addition of an antiangiogenic therapy will be spared the side effects of the treatment and the response of likely-responders will be optimized.

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Table 1

Demographics, tumor characteristics and pCR outcome.

Patient Characteristics by pCR status	Did not attain pCR, N (%) ¹	Attained pCR, N (%) ²	P ³
Triple Negativity			
Other (ER+ or PR+ or HER2+)	17 (77%)	6 (50%)	0.10
Triple negative	5 (23%)	6 (50%)	
Hormone Receptor Status			
ER or PR or both are positive	16 (73%)	4 (33%)	0.026
Both ER and PR are negative	6 (27%)	8 (67%)	
HER2/Neu status			
Positive	5 (23%)	2 (17%)	0.68
Negative	17 (77%)	10 (83%)	
Grade			
I, II	11 (50%)	3 (25%)	0.16
III	11 (50%)	9 (75%)	
Cancer Type			
Ductal Carcinoma	14 (64%)	11 (92%)	0.077
Lobular/Poorly differentiated	8 (36%)	1 (8%)	
Surgery			
Mastectomy	18 (82%)	8 (67%)	0.32
Lumpectomy	4 (18%)	4 (33%)	
Race [†]			
African American	3 (15%)	5 (42%)	0.092
Caucasian	17 (85%)	7 (58%)	
Age Group			
Under 50 years	12 (55%)	6 (50%)	0.80
50 years or Older	10 (45%)	6 (50%)	
BMI Group			
BMI <30 (not obese)	17 (77%)	7 (58%)	0.25
BMI ≥30 (obese)	5 (23%)	5 (42%)	

¹Number and percent of 22 subjects who did not attain pCR.²Number and percent of 12 subjects who attained pCR.³P values are from two-sided chi-square tests.[†]excluding 1 Hispanic and 1 Asian, both of whom did not attain pCR.

Table 2

Serum proteins at baseline.

Serum Protein	LOD ¹	pCR	Median ²	Lower Quartile ²	Upper Quartile ²	Minimum ²	Maximum ²	P ³
ANG-1	33.4	no	35,200	28,400	39,500	16,100	63,400	0.49
		yes	33,800	27,000	37,700	22,000	39,100	
ANG-2	14.5	no	6,350	5,170	7,970	3,520	11,900	0.69
		yes	5,540	4,540	8,390	2,770	19,700	
bFGF	33.3	no	<33.3	<33.3	68.7	<33.3	313.0	0.045
		yes	73.4	36.7	137.0	<33.3	290.0	
IL-1a	6.2	no	15.5	<6.2	27.2	<6.2	67.0	0.34
		yes	28.2	8.8	40.4	<6.2	75.3	
MMP-9	6.8	no	4,380	2,400	5,990	715	8,750	0.64
		yes	4,230	2,940	6,980	1,510	8,430	
PDGF-BB	6.4	no	17,900	14,700	20,900	10,200	30,700	0.89
		yes	16,700	15,400	20,300	14,600	28,700	
PECAM-1	79.9	no	1,640	1,100	2,080	353	3,440	0.47
		yes	1,950	894	2,310	623	4,030	
Tie-2	91.2	no	416	303	467	202	990	0.0013
		yes	617	514	870	359	1,330	
VEGF	10.1	no	1,180	702	2,290	170	9,350	0.18
		yes	2,010	1,080	4,450	426	8,700	
VEGF R2	8.2	no	4,160	3,590	5,000	2,410	8,240	0.22
		yes	4,750	3,970	5,940	3,280	7,330	

¹Limit of detection, in picograms per milliliter (pg/mL).²All quantiles are expressed in pg/mL. Values that are preceded by a "<" symbol indicates quantiles that were less than the LOD.³Wilcoxon rank-sum *P* values, 2-sided at alpha=0.05; significant values are shown in bold.

Table 3

Maximum post-baseline values after initiation of treatment.

Protein Label	Attained pCR?	Maximum post-baseline values				Differences, Maximum – Baseline			
		Median ¹	Lower Quartile ¹	Upper Quartile ¹	p ²	Median ¹	Lower Quartile ¹	Upper Quartile ¹	p ²
ANG-1	no	43,700	35,400	50,100	1.00	5,490	-930	13,900	0.21
	yes	42,200	38,200	46,300		11,100	4,890	19,800	
ANG-2	no	7,790	5,450	10,800	0.59	1,000	-21.6	3,540	0.75
	yes	8,340	6,170	11,300		2,430	-329.0	3,640	
bFGF	no	75.7	38.7	83.6	0.037	9.4	0.0	42.1	0.43
	yes	105.0	75.3	156.0		5.9	-15.1	35.1	
IL-1a	no	23.7	14.1	31.7	0.25	6.5	1.4	15.2	1.00
	yes	28.1	18.9	48.5		7.9	-0.0	14.1	
MMP-9	no	6,660	5,760	7,580	0.11	2,050	308	5,080	0.91
	yes	7,460	6,850	8,280		1,980	247	4,300	
PDGF-BB	no	19,400	16,300	23,400	0.64	1,540	-345	2,840	0.89
	yes	19,900	16,900	24,200		1,430	-2,290	3,250	
PECAM-1	no	1,950	1,640	2,840	0.97	623	-16.8	936	0.67
	yes	2,010	1,530	3,450		401	-317	1,130	
Tie-2	no	517	431	943	0.14	198	71.3	475	0.37
	yes	838	514	1150		114	50.9	294	
VEGF	no	324	272	381	0.31	-875	-2,000	-370	0.28
	yes	362	303	404		-1,330	-4,140	-563	
VEGF R2	no	5,790	5,010	6,580	0.61	1,520	123	2,840	0.26
	yes	5,420	4,790	6,230		532	290	1,570	

¹ Medians and quartiles are in units of pg/mL² P-values are from 2-sided Wilcoxon rank-sum tests.

Table 4

logistic-regression analysis of baseline serum proteins in the presence of ER/PR status.

Serum Protein	Adjusted OR ¹	95% Confidence Interval	P	ER/PR status	Adjusted OR ²	95% Confidence Interval	P
ANG-1	0.85	0.35–2.07	0.72	–/–	5.02	1.07–23.7	0.041
ANG-2	1.11	0.52–2.35	0.79	–/–	5.26	1.14–24.2	0.033
bFGF	1.22	0.73–2.04	0.46	–/–	4.62	0.97–22.1	0.055
IL-1a	3.05	0.99–9.43	0.053	–/–	9.32	1.53–56.9	0.016
MMP-9	1.07	0.26–4.44	0.93	–/–	5.24	1.10–25.0	0.038
PDGF-BB	1.16	0.48–2.79	0.74	–/–	5.64	1.17–27.1	0.031
PECAM-1	1.29	0.46–3.59	0.63	–/–	4.98	1.06–23.4	0.042
Tie-2	3.70	1.22–11.3	0.021	–/–	3.92	0.69–22.4	0.124
VEGF	1.72	0.92–3.23	0.091	–/–	7.61	1.36–42.6	0.021
VEGF-R2	1.56	0.56–4.35	0.40	–/–	5.05	1.08–23.6	0.040

¹ Adjusted odds ratio favoring pCR with a one-unit increase in baseline level of the indicated serum protein, adjusting for the presence of ER/PR status in the logistic-regression model. Protein concentrations were normalized so that one unit of increase in baseline level is equal to the protein's interquartile range (IQR) at baseline.

² Adjusted odds ratio favoring pCR in subjects with ER/PR=“–/–” compared to subjects with at least one “+”, adjusting for the presence of baseline serum-protein levels in the logistic-regression model.