Cell Adhesion Molecules, Vascular Endothelial Growth Factor, and Basic Fibroblast Growth Factor in Patients with Non–Small Cell Lung Cancer Treated with Chemotherapy with or without Bevacizumab—an Eastern Cooperative Oncology Group Study

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Abstract

Background: E4599 was a phase II/phase III trial, in which 878 patients with advanced non–small cell lung cancer were randomized to carboplatin + paclitaxel (PC arm) or PC + bevacizumab (BPC arm). Survival and progression-free survival were superior on the BPC arm. The rationale for markers used in this correlative study was based on elevated vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), soluble intercellular adhesion molecule (ICAM) and E-selectin in a variety of malignances and changes in response to endothelial cell apoptosis.

Materials and Methods: Prospective correlates included measurements of pretreatment plasma VEGF, as well as pretreatment and week 7, bFGF, ICAM, and E-selectin. Low and high levels were defined as less than or equal to or more than the median.

Results: E-selectin ($P < 0.0001$) showed a decrease and bFGF showed an increase ($P = 0.004$) from baseline at week 7, which were similar in both arms. Baseline ICAM showed significant associations with response and survival in both groups. Patients with low baseline ICAM had a higher response rate (32% versus 14%; $P = 0.02$), better overall survival ($P = 0.00005$), and better 1-year survival (65% versus 25%) than those with high ICAM, respectively, regardless of treatment arm. Patients with high VEGF levels were more likely to respond to BPC compared with PC, but this was not predictive of survival. The results also suggest a benefit from bevacizumab for patients with low baseline ICAM levels (53% reduction in the progression-free survival hazard rate).

Conclusions: In this study, baseline ICAM levels were prognostic for survival and predictive of response to chemotherapy with or without bevacizumab. VEGF levels were predictive of response to bevacizumab but not survival.

Lung cancer is the leading cause of cancer-related deaths in the United States (1). Non–small cell lung cancer comprises >80% of lung cancer cases. The majority of patients present with advanced stage disease where the 5-year survival is <5%. In patients with advanced non–small cell lung cancer with a good performance status, combination chemotherapy provides a modest improvement in survival (2). Clinical prognostic factors for survival in this patient population include sex, performance status, and stage of disease (3). Although many biological markers have been evaluated for their ability to predict prognosis in advanced disease, these studies have uniformly been retrospective (4).

Angiogenesis is a biological event of critical importance in tumor growth and progression. A number of angiogenic factors have been identified, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF; ref. 5). Recently, the addition of a humanized monoclonal antibody targeting the VEGF (bevacizumab) to chemotherapy has been shown to increase response rates, progression-free survival (PFS) and overall survival in nonsquamous advanced non–small cell lung cancer when compared with chemotherapy alone (6). Although predictive factors for response to another class of targeted therapies in lung cancer, epidermal growth factor receptor inhibitors, have been extensively reported (7), no predictive factors for response to VEGF pathway inhibitors have been found (8).

Intercellular adhesion molecule-1 (ICAM-1) is a single-chain cell surface glycoprotein and is expressed constitutively at low levels on endothelial cells, some lymphocytes, and monocytes (9). The serum or plasma concentration of soluble ICAM-1 (called ICAM hereon) in patients with a variety of malignancies is associated with disease progression or prognosis, including breast cancer (10), colorectal cancer (11), and malignant

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Table 1. Summary of plasma E-selectin (ng/mL), bFGF (pg/mL), ICAM (ng/mL), and VEGF (pg/mL)

Assay	Treatment	Time	*n*	Min.	Median	Max.
E-selectin	Combined	Pre Rx	149	11.5	38.8	158
E-selectin	Combined	7+ wk	112	10.2	32.5	117
E-selectin	PC	Pre Rx	72	11.5	40.4	158
E-selectin	BPC	Pre Rx	77	12.3	38.3	115
E-selectin	PC	7+ wk	56	12.0	33.0	117
E-selectin	BPC	7+ wk	56	10.2	29.3	87
bFGF	Combined	Pre Rx	150	0.4	7.4	168
bFGF	Combined	7+ wk	113	1.5	8.4	192
bFGF	PC	Pre Rx	73	0.4	7.9	43.7
bFGF	BPC	Pre Rx	77	1.2	7.0	168
bFGF	PC	7+ wk	57	2.7	9.2	192
bFGF	BPC	7+ wk	56	1.5	7.7	35.9
ICAM	Combined	Pre Rx	150	105	260	1390
ICAM	Combined	7+ wk	113	156	257	1075
ICAM	PC	Pre Rx	73	149	271	1390
ICAM	BPC	Pre Rx	77	105	249	483
ICAM	PC	7+ wk	57	171	271	1075
ICAM	BPC	7+ wk	56	156	245	664
VEGF	Combined	Pre Rx	166	12.0	35.7	839
VEGF	PC	Pre Rx	79	12.0	38.7	839
VEGF	BPC	Pre Rx	87	12.0	33.7	491

NOTE: The summaries are given for the two treatment arms combined and for the arms separately (PC and BPC). For VEGF, 17 cases coded as <12.5 were given the value 12 and seven cases coded as >445 were given the value 446 for this summary.
Abbreviation: Pre Rx, Pre-treatment.

melanoma (12). E-selectin is a transmembrane glycoprotein that is expressed only on endothelial cells and only after activation by inflammatory cytokines or endotoxin (13) and can be measured in soluble form (soluble E-selectin, called E-selectin hereon). Because the primary target of bevacizumab is the endothelial cell, we hypothesized that damage and/or apoptosis of vascular endothelial cells will be associated with the release of endothelial cell–specific markers in plasma (ICAM and E-selectin). The rationale for this hypothesis is supported by the detection of elevated levels of certain of these markers in thrombotic thrombocytopenic purpura (14, 15), a disorder characterized by endothelial cell apoptosis, as well as in hypercholesterolemia (16), another disorder characterized by endothelial cell dysfunction. Similarly, elevated levels of E-selectin reflect early endothelial dysfunction in patients with glucose intolerance (17).

We and others have measured ICAM and E-selectin in early-phase trials of antivascular or antiangiogenic agents. In two phase I studies, we evaluated the levels of ICAM and E-selectin at serial time points after administration of either a vascular-targeting agent (combretastatin A4 phosphate; ref. 18) or an agent targeting VEGF receptor tyrosine kinase activity (SU5416; ref. 19). We showed an acute rise in ICAM (progressive increase from pretreatment to 1 and 24 h) after administration of combretastatin and a more prolonged increase with SU5416. Alternatively, for E-selectin, we saw a progressive increase for patients receiving SU5416 but not combretastatin. Devore and colleagues measured plasma levels of E-selectin and found an increase in patients receiving the antiangiogenic agent CM-101 (20). The acute increase of these adhesion molecules suggested a real pharmacodynamic effect of the agents tested, whereas the more chronic increase may have simply reflected progressive disease.

Based on the above, the Eastern Cooperative Oncology Group Lung Cancer Biology subcommittee planned a prospective biomarker assessment of VEGF, bFGF, ICAM, and E-selectin within the E4599 randomized trial evaluating chemotherapy ± bevacizumab in patients with advanced lung cancer to determine the prognostic and predictive worth of these markers.

Materials and Methods

In this randomized phase II/phase III trial, treatment assignments were designed to achieve balance between the two treatment groups using the following stratification factors: measurable versus nonmeasurable disease, prior radiation versus no prior radiation, prior weight loss of <5% versus ≥5%, and stage IIIB with pleural effusion versus stage IV or recurrent disease. The primary study end point was overall survival and has been reported (6). The protocol was approved by the institutional review boards of all participating institutions and was carried out in accordance with the Declaration of Helsinki, current Food and Drug Administration Good Clinical Practices, and local ethical and legal requirements.

Patients were randomized to receive paclitaxel (200 mg/m^2) and carboplatin (AUC, 6 each) given i.v. on day 1 without (carboplatin + paclitaxel, PC) or with bevacizumab (15 mg/kg, i.v.) on day 1 (PC + bevacizumab, BPC). Chemotherapy was repeated every 21 days up to a total of six cycles, unless there was evidence of disease progression or patient intolerance. Patients randomized to BPC were continued on bevacizumab monotherapy every 3 weeks until evidence of disease progression or unacceptable toxicity.

The first objective of this correlative study was to determine if differences in the pretreatment levels of plasma VEGF predict response to chemotherapy ± bevacizumab. For the correlative study, 80 plasma samples would be available from each arm (first 160 consecutive patients enrolled on study). Subjects were divided into two groups based on their baseline VEGF levels using the median value as the cutoff. For this analysis, response was dichotomized into responders (best response of partial or complete response per RECIST criteria) and nonresponders. Within each of the VEGF groupings, a Fisher exact test with a two-sided 5% type I error rate was used to detect an association between response and treatment. With the planned sample size, this test would have 80% power to detect a difference in response rates of 15% on PC versus 46% on BPC. The secondary objective of this correlative study was to perform exploratory analysis of baseline levels and serial

Table 2. Association between baseline levels, with clinical characteristics and response for all patients

	E-selectin		bFGF		ICAM		VEGF	
	Low	High	Low	High	Low	High	Low	High
% Male	55	69	59	64	65	57	59	60
% Age ≥65	44	36	44	37	40	41	43	39
% PS 0	32	45	45	32	41	36	41	30
% Response	28	20	24	23	32*	14*	29	21

NOTE: Low and high levels are defined as less than or equal to the median value and more than the median. There are 10 cases in the E-selectin, bFGF, and ICAM analyses and 15 in the VEGF analyses that did not have measurable disease. Patients without measurable disease are excluded from the response comparisons.
*P = 0.01; P > 0.05 for all other low versus high comparisons.

Table 3. Response rates by treatment assignment within factor levels

	PC	BPC	*P*
E-selectin low	22.6	32.4	0.42
E-selectin high	10.8	29.4	0.07
bFGF low	18.8	28.9	0.41
bFGF high	13.5	33.3	0.09
ICAM low	22.6	40.0	0.13
ICAM high	10.5	19.4	0.33
VEGF low*	29.0	28.6	1.00
VEGF high*	7.7	33.3	0.01

NOTE: Low and high are defined by median cutoffs for each of the factors. The analyses are restricted to patients with measurable disease. The *P* values are from Fisher's exact tests for a treatment difference in each subgroup.
*Logistic regression treatment interaction test; $P = 0.04$.

changes in ICAM, E-selectin, and bFGF. These factors are also categorized as high/low using median cutoffs for all analyses. Overall survival is defined to be time from randomization to death from any cause, censored at the date of last contact. PFS is time from randomization to documented progression per RECIST or death, censored at the date of the last documented disease evaluation for patients without a PFS event reported. The Kaplan-Meier method is used to estimate survival and PFS distributions. Log-rank tests are used for univariate comparisons of survival and PFS end points. Cox proportional hazards models are used to estimate hazard ratios in regression models. Tests for differences in treatment effects between marker groups use interaction Wald tests from logistic regression models (for response) and Cox models (for PFS and survival). All *P* values are two-sided. Analyses of individual factors used all cases with values for that factor, and models containing more than one factor included only cases with data on all factors in the model. Because of the exploratory nature of the analyses (other than the primary objective), no adjustment for multiple testing was made.

Plasma samples were collected from patients before cycle 1 and after completion of cycle 2 (precycle 3; termed hereon as "week 7" samples). Pretreatment samples were analyzed for levels of ICAM, E-selectin, bFGF, and VEGF using commercially available ELISA kits (R&D Systems). Same factors were measured at week 7 except for VEGF (repeated measures of VEGF in the setting of treatment with bevacizumab show consistently high levels due to binding of antibody to ligand and increase in half life.) Three milliliters of peripheral blood were drawn into citrated Vacutainer tube and mixed immediately by inverting the tube 10 to 15 times. Samples were then centrifuged within 30 min of collection for 10 min at 4°C at 3,000 × *g*. Plasma was removed and transferred to cryogenic storage tubes. Samples were stored immediately stored at -70°C. They were then shipped on dry ice to University Hospitals Case Medical Center (A. Dowlati) for analysis. Samples were run after the first thaw only, in duplicate, and the average was recorded. The lower limits of detection for ICAM, E-selectin, VEGF, and bFGF were 0.35 ng/mL, 0.1 ng/mL, 12.5 pg/mL, and 1.0 pg/mL, respectively. The intraassay coefficient of variation was 4.6%, 5%, 4%, and 4.3%, respectively.

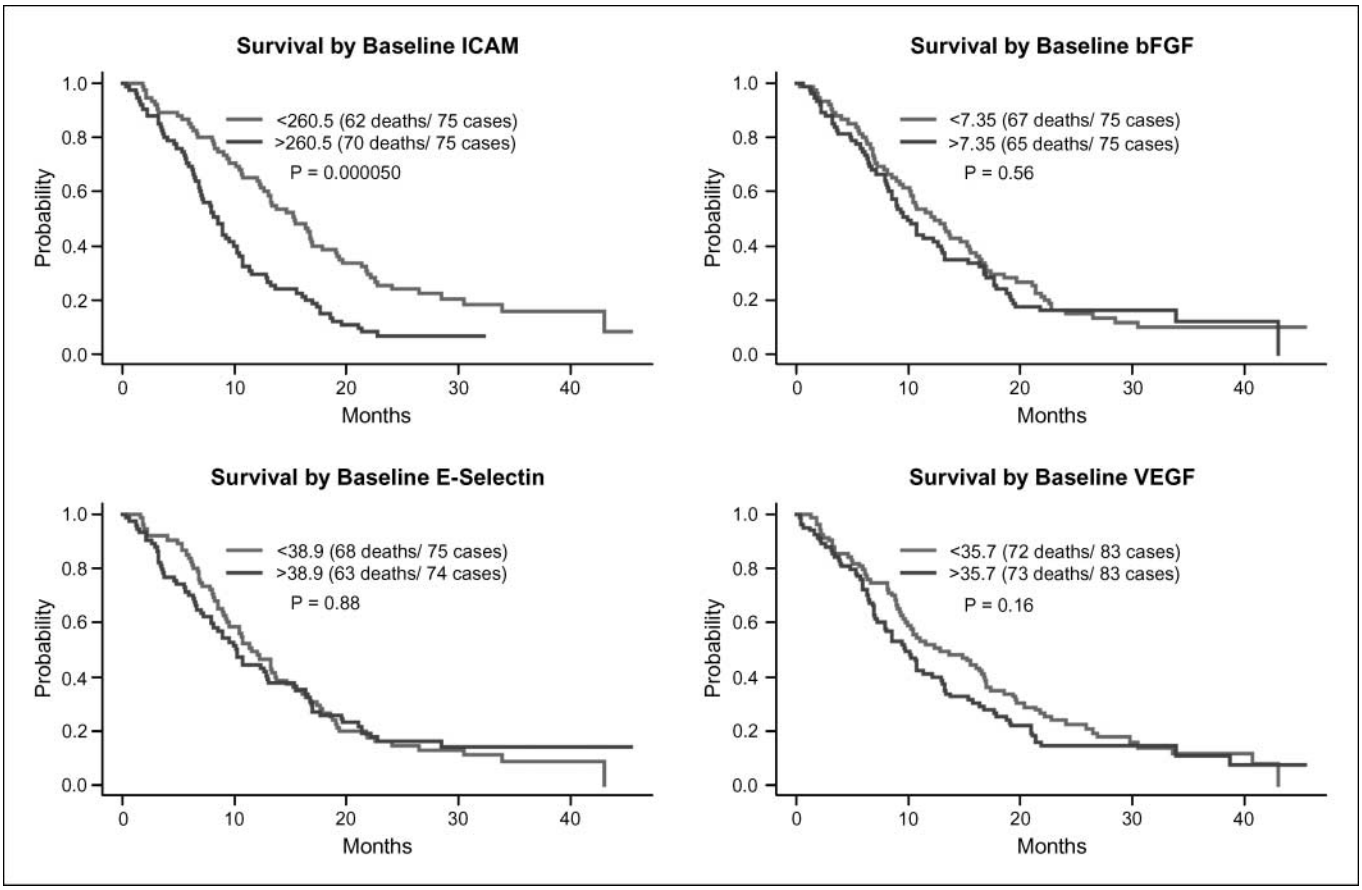

Fig. 1. Survival based on baseline factor levels.

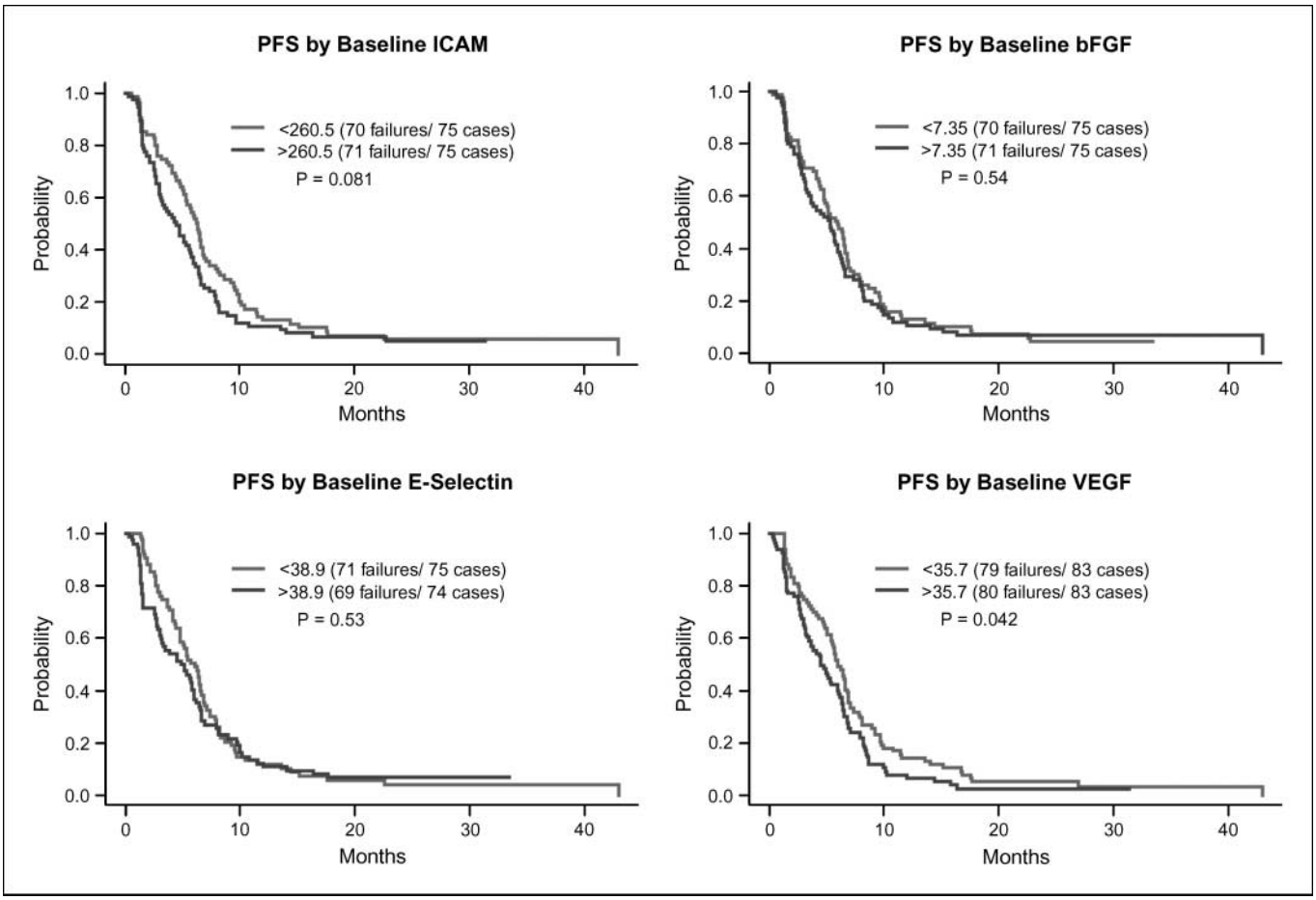

Fig. 2. PFS based on baseline levels.

Results

Measurement of factor levels at baseline and week 7. Table 1 summarizes the distribution of the measurements on these factors. The baseline ICAM levels are significantly higher on PC than BPC arm (P = 0.02, Wilcoxon rank sum test). The distributions of the baseline E-selectin, bFGF, and VEGF levels are not significantly different between the treatment arms. E-selectin shows a significant decrease from baseline at week 7 (P < 0.0001, Wilcoxon signed-rank test), and bFGF shows a significant increase from baseline (P = 0.004) in the combined sample (Supplementary Fig. S1). In both cases, the magnitude of the change is not significantly different between the treatment arms (Wilcoxon rank sum test). The change from baseline in ICAM is not significant.

Association between factor levels, clinical characteristics and response rate among all patients. Table 2 examines the association among factor levels at baseline with selected baseline clinical characteristics and with objective tumor response rate. Throughout, low and high levels are defined as less than or equal to the median value and more than the median. There are no correlations between any of the baseline factor levels, gender, age of ≥65 or <65, or performance status. Only ICAM shows a significant association with response. In patients with a low ICAM level, the response rate was 32% versus 14% in the high ICAM group (P = 0.02).

Association between factor levels and response according to treatment assignment. Table 3 examines response by treatment assignment (BPC or PC) separately for groups defined by low and high baseline levels. The primary end point of the correlative study which was to determine if baseline VEGF levels is predictive of response to chemotherapy ± bevacizumab was met. The logistic regression interaction test, which tests whether the effect of treatment is different within the low and high levels, has P = 0.04 for VEGF measurements, suggesting that patients with high baseline VEGF levels are more likely to have an increased probability of response with the addition of bevacizumab (33% response on BPC arm versus 7.7% on PC arm, P = 0.01) than those with low VEGF levels (28.6% response on BPC arm versus 29% on PC arm, P = not significant). The interaction tests are not significant for any of the other factors.

Association between baseline factor level and progression-free or overall survival among all patients. Figures 1 and 2 give survival and PFS by baseline factor levels. Baseline ICAM seems to be a significant prognostic factor for survival (P = 0.00005). This difference seems to be consistent for the two treatment arms (data not shown). Patients with a low baseline ICAM have a 1-year survival of 65% versus 25% for patients with high ICAM levels. Overall survival was not associated with any other factor. Baseline VEGF was, however, a significant predictor of PFS. Patients with a low VEGF level had a better PFS (median,

6.0 months) compared with patients with a high level (median, 4.5 months; $P = 0.04$). Other comparisons were not statistically significant.

For survival, the variables baseline ICAM (estimated hazard ratio for high/low, 1.83; $P = 0.001$), performance status (1/0 estimated hazard ratio, 1.73; $P = 0.005$), sex (male/female estimated hazard ratio, 1.60; $P = 0.01$), bone involvement (estimated hazard ratio, 2.15; $P = 0.00007$), liver involvement (estimated hazard ratio, 1.80; $P = 0.006$), nonrecurrent (versus recurrent, estimated hazard ratio, 1.91; $P = 0.01$), and adrenal involvement (estimated hazard ratio, 1.75; $P = 0.02$) were all significant in a joint proportional hazards model. None of the variables baseline E-selectin, baseline bFGF, or baseline VEGF added significantly to this model.

For PFS, the variables VEGF (estimated hazard ratio, 1.53; $P = 0.009$), bone involvement (estimated hazard ratio, 1.48; $P = 0.03$), recurrent disease (versus nonrecurrent stage IV, estimated hazard ratio, 0.62; $P = 0.03$), and nonrecurrent stage IIIB (versus nonrecurrent stage IV, estimated hazard ratio, 0.46; $P = 0.04$) were all significant in a joint model. None of the variables baseline E-selectin, baseline bFGF, or baseline ICAM added significantly to this model.

Predictive value of baseline factor levels or changes in levels on the benefits of progression-free or overall survival with bevacizumab. Table 4 examines overall survival and PFS by treatment separately for groups defined by low and high baseline levels and for groups defined by low and high levels of changes from baseline (week 7 minus baseline levels). As can be seen in this table, the hazard ratio (PC/BPC) of death or disease progression is >1 in almost all subcategories, indicating the superiority of BPC over PC. There are two exceptions: patients with high baseline ICAM levels have a hazard ratio of 1.0 for PFS (95% confidence interval, 0.62-1.6), whereas those with low baseline ICAM levels have a hazard ratio of 2.14 for PFS (95% confidence interval, 1.31-3.48; $P < 0.001$ from Cox partial likelihood Wald tests for a treatment difference in each subgroup). The second exception is that patients with a drop in E-selectin of >5.35 ng/mL have a hazard ratio of 0.94 for survival (95% confidence interval, 0.54-1.63), whereas patients with a drop of E-selectin of ≤5.35 have a hazard ratio of 1.98 for overall survival (95% confidence interval, 1.1-3.57; $P = 0.02$). Here (and elsewhere), the interpretation of differences in change from baseline is limited to patients continuing on study and progression-free for at least two cycles. Tests for treatment by factor interactions, which test whether the effect of treatment is different within the low and high levels, are significant for the difference in E-selectin levels for survival ($P = 0.05$) and for baseline ICAM for PFS ($P = 0.04$), although these interaction tests do not have good power for the small sample sizes here. The results suggest a substantial benefit from bevacizumab for patients with decreases in E-selectin levels of <5.35 (49% reduction in the mortality hazard rate) or with low baseline ICAM levels (53% reduction in the PFS hazard rate).

Discussion

A plethora of tumor markers have been reported in recent years. Significant variability in methodologic assessment of markers exists, and therefore, recent reporting recommendations for tumor marker prognostic studies have been published (21, 22). Our study follows those recommendations. In this prospective study, ICAM was a strong independent prognostic factor for overall survival. This is in concordance with initial body of evidence relating to ICAM in other malignancies (10–12). Whereas the source of soluble ICAM has not been totally elucidated, it is felt that endothelial cells are an important source. *In vitro* studies using cultured endothelial cells established that soluble ICAM may simply reflect ICAM expression on these cells (23). Therefore the better survival of patients with lower ICAM levels may reflect the tumor angiogenic load, with highly angiogenic tumors having higher levels and worse prognosis. The magnitude of difference in survival in patients categorized as having low ICAM levels (≤260.5 ng/mL) as opposed to high levels is quite significant with a 1-year survival of 65% versus 25%, respectively. In addition, ICAM was predictive for response to chemotherapy with or without bevacizumab. Patients with low ICAM levels had an increased probability of response to chemotherapy. This is the first reported study showing such a finding. Although we also had hypothesized that an increase in E-selectin and ICAM would be seen with treatment, we saw no significant change in ICAM at week 7 compared with baseline and actually also saw a statistically significant decrease in E-selectin that was similar on both arms.

VEGF levels on the other hand were not prognostic for overall survival. This is in concordance with data from two smaller studies, where serum VEGF was not prognostic for survival in early stage non–small cell lung cancer (24) nor did it correlate with the presence of distant metastases (25). VEGF levels were, however, predictive of response to bevacizumab in our study, and therefore, the primary end point of this correlative study was reached. Patients with high levels of baseline plasma VEGF (>35.7 pg/mL) had an increased

Table 4. Hazard ratios (PC/BPC) and 95% confidence intervals for the effect of treatment on survival and PFS

	Survival			PFS		
	PC/BPC	LCL	UCL	PC/BPC	LCL	UCL
E-selectin low	1.12	0.69	1.82	1.35	0.84	2.18
E-selectin high	1.50	0.91	2.48	1.70	1.05	2.75
bFGF low	1.35	0.83	2.19	1.84	1.14	2.96
bFGF high	1.17	0.71	1.92	1.22	0.76	1.96
ICAM low	1.39	0.84	2.30	2.14*	1.31	3.48
ICAM high	0.90	0.56	1.44	1.00*	0.62	1.60
VEGF low	1.21	0.76	1.93	1.45	0.92	2.28
VEGF high	1.33	0.84	2.12	1.34	0.86	2.08
Δ E-selectin low	1.98*	1.10	3.57	2.17	1.24	3.79
Δ E-selectin high	0.94*	0.54	1.63	1.41	0.82	2.44
Δ bFGF low	1.32	0.76	2.30	1.82	1.06	3.12
Δ bFGF high	1.51	0.83	2.72	1.52	0.86	2.66
Δ ICAM low	1.17	0.68	2.03	1.57	0.91	2.72
Δ ICAM high	1.65	0.91	3.00	2.03	1.17	3.55

NOTE: Low and high are defined by median cutoffs for each of the factors, and Δ denotes the difference between the week 7 and baseline values. The treatment difference is significant ($P < 0.05$) in a group if the confidence interval does not contain 1. The analyses using differences from baseline are "landmark" analyses, because only cases with follow-up samples are included.
Abbreviations: LCL, lower 95% confidence limits on the hazard ratio; UCL, upper 95% confidence limits on the hazard ratio.
*Cox model treatment interaction tests; $P \leq 0.05$.

probability of response to the BPC arm. The response rate in patients with low VEGF levels (≤35.7 pg/mL) was similar in PC and BPC arms. It is important to note, however, that VEGF levels were not predictive of the survival benefit afforded by the addition of bevacizumab to chemotherapy, and therefore, VEGF measurements have no clinical utility in this setting. This discrepancy between the ability of plasma VEGF to predict response, but not survival, may simply indicate that the improvement in survival seen with the addition of bevacizumab may not be related to the ability of this agent to increase response rate when added to chemotherapy.

In almost all subgroups, the hazard of death and progression were higher on the PC arm compared with BPC arm indicating the superiority of BPC over PC. For two groups, this hazard ratio was near or above 2: patients with low baseline ICAM and patients with a drop in E-selectin of ≤5.35 ng/mL at week 7. Tests for treatment by factor interactions, which test whether the effect of treatment is different within the low and high levels, were significant for the difference in E-selectin levels (week 7 minus baseline) for survival ($P = 0.05$) and for baseline ICAM for PFS ($P = 0.04$). It is important to note that these interaction tests do not have good power given the small sample sizes. Nevertheless, the results suggest a substantial benefit from bevacizumab for patients with decreases in E-selectin levels of <5.35 ng/mL (49% reduction in the mortality hazard rate) or with low baseline ICAM levels (53% reduction in the PFS hazard rate). We had previously observed an increase in E-selectin in patients (of whom all progressed on therapy) receiving the VEGF receptor inhibitor SU5416. Our findings in E4599 related to E-selectin may simply suggest the patients with significant progression of their disease (i.e., more aggressive tumor biology) are less likely to benefit from bevacizumab-based treatments. The finding that low ICAM level patients had a 53% reduction in PFS hazard rate when treated with bevacizumab may also indicate again that patients with lower tumor burden are those most likely to benefit from bevacizumab-based therapy. Other studies have looked at predictive factors on the benefit of bevacizumab and failed to show any predictive value. Jubb et al. looked at tissue expression of VEGF-A, thrombospondin-2, and microvessel density and found no predictive benefit in patients with metastatic colon cancer treated with 5-fluorouracil–based therapy ± bevacizumab (8). Another group failed to show a statistically significant relationship between mutations of k-ras, b-raf, or p53 and the increase in median survival associated with the addition of bevacizumab to IFL in metastatic colorectal cancer (26). Of importance, unlike our trial, which was prospectively planned, all other studies were retrospective.

The search for predictive markers for response and benefit from antiangiogenic agents continues. One promising marker is circulating endothelial progenitor cells. Levels of these progenitor cells decrease in response to antiangiogenic agents. Another marker under active investigation is the soluble VEGF receptor-2. The utility of these markers remain under active investigation (27, 28).

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References

1. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. CA Cancer J Clin 2005;55:10–30.
2. American Society of Clinical Oncology treatment of unresectable non-small-cell lung cancer guideline: update 2003. J Clin Oncol 2004;22:330–53.
3. Paesmans M, Sculier JP, Libert P, et al. Prognostic factors for survival in advanced non-small-cell lung cancer: univariate and multivariate analyses including recursive partitioning and amalgamation algorithms in 1,052 patients. The European Lung Cancer Working Party. J Clin Oncol 1995;13:1221–30.
4. Koutsami MK, Gorgoulis VG, Kastrinakis NG, et al. Prognostic factors in non-small cell lung carcinoma. Anticancer Res 2002;22:347–74.
5. Folkman J. Angiogenesis. Annu Rev Med 2006;57:1–18.
6. Sandler AB, Gray R, Perry M, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small cell lung cancer. N Engl J Med 2006;355:2542–50.
7. Bunn PA, Dziadziuszko R, Vallera-Garcia M, et al. Biological markers for non-small cell lung cancer patient selection for epidermal growth factor receptor tyrosine kinase inhibitor therapy. Clin Cancer Res 2006;15:3652–6.
8. Jubb AM, Hurwitz HI, Bai W, et al. Impact of vascular endothelial growth factor-A expression, thrombospondin-2 expression, and microvessel density on the treatment effect of bevacizumab in metastatic colorectal cancer. J Clin Oncol 2006;24:217–27.
9. Witkowska AM, Borawska MH. Soluble intercellular adhesion molecule-1 (sICAM-1): an overview. Eur Cytokine Netw 2004;15:91–8.
10. Zhang GJ, Adachi L. Serum levels of soluble intercellular adhesion molecule-1 and E-Selectin in metastatic breast carcinoma: correlations with clinicopathological features and prognosis. Int J Oncol 1999;14:71.
11. Alexiou D, Karayiannakis AJ, Syrigos KN, et al. Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival, and tumor surgery. Eur J Cancer 2001;37:2392.
12. Giavazzi R, Chirvi RG, Garofalo A, et al. Soluble intercellular adhesion molecule-1 is released by human melanoma cells and is associated with tumor growth in nude mice. Cancer Res 1992;52:2628.
13. Bevilacqua MP, Nelson RM. Selectins. J Clin Invest 1993;91:379–87.
14. Wada H, KanekoT, Ohiwa M, et al. Increased levels of vascular endothelial cell markers in thrombotic thrombocytopenic purpura. Am J Hematol 1993;44:101–5.
15. Dang CT, Magid MS, Weksler B, et al. Enhanced endothelial cell apoptosis in splenic tissues of patients with thrombotic thrombocytopenic purpura. Blood 1999;93:1264–70.
16. Wada H, Mori Y, Kaneko T, et al. Elevated levels of vascular endothelial cell markers in patients with hypercholesterolemia. Am J Hematol 1993;44:112–6.
17. Ferri C, Desideri G, Bellini C, et al. Early activation of vascular endothelium in nonobese, nondiabetic essential hypertensive patients with multiple metabolic abnormalities. Diabetes 1998;47:660–7.
18. Dowlati A, Cooney M, Robertson K, et al. A phase I pharmacokinetic and translational study of the novel vascular targeting agent combretastatin a-4 phosphate on a single-dose intravenous schedule in patients with advanced cancer. Cancer Res 2002;62:3408–16.
19. Dowlati A, Robertson K, Radovotyicivh T, et al. Novel phase I dose de-escalation design trial to determine the biological modulatory dose of the antiangiogenic agent SU5416. Clin Cancer Res 2005;11:7938–44.
20. Devore RF, Hellerquist CG, Wakefield GB, et al. Phase I study of the antineovascularization drug CM101. Clin Cancer Res 1997;3:365–72.
21. McShane LM, Altman DG, Saubrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor prognostic studies. J Clin Oncol 2005;23:9067–72.
22. Altman DG, Riley RD. Primer: an evidence-based approach to prognostic markers. Nat Clin Prac Oncol 2005;2:466–72.
23. Leeuwenerg JFM, Smeets EF, Neefjes JJ, et al. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cell *in vitro*. Immunology 1992;77:543.
24. Imoto H, Osaki T, Taga S, et al. Vascular endothelial growth factor expression in non-small cell lung cancer: prognostic significance in squamous cell carcinoma. J Thorac Cardiovasc Surg 1998;115:1007–114.
25. Takiqawa N, Seqawa Y, Fujimoto N, et al. Elevated vascular endothelial growth factor in sera of patients with lung cancer. Anticancer Res 1998;18:1251–4.
26. Ince WL, Jubb AM, Holden SN, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. J Natl Cancer Inst 2005;97:981–9.
27. Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res 2005;65:671–80.
28. Shaked Y, Bocci G, Munoz R, et al. Cellular and molecular surrogate markers to monitor targeted and non-targeted antiangiogenic drug activity and determine optimal biologic dose. Curr Cancer Drug Targets 2005;5:551–9.

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