Improved tumor vascularization after anti-VEGF therapy with carboplatin and nab-paclitaxel associates with survival in lung cancer

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Addition of anti-VEGF antibody therapy to standard chemotherapies has improved survival and is an accepted standard of care for advanced non–small cell lung cancer (NSCLC). However, the mechanisms by which anti-VEGF therapy increases survival remain unclear. We evaluated dynamic CT-based vascular parameters and plasma cytokines after bevacizumab alone and after bevacizumab plus chemotherapy with carboplatin and nab-paclitaxel in advanced NSCLC patients to explore potential biomarkers of treatment response and resistance to this regimen. Thirty-six patients were enrolled in this study. The primary end point was 6-mo progression-free survival rate, which was 74% (95% CI: 57, 97). This regimen has a promising overall response rate of 36% and median time to progression of 8.5 (6.0, 38.7) mo and overall survival of 12.2 (9.6, 44.1) mo. We found that anti-VEGF therapy led to a sustained increase in plasma PlGF, a potential pharmacodynamic marker. We also found that higher levels of soluble VEGFR1 measured before starting bevacizumab with chemotherapy were associated with worse survival, supporting its potential role as biomarker of treatment resistance. Our imaging biomarker studies indicate that bevacizumab-based treatment—while reducing blood flow, volume, and permeability in the overall population—may be associated with improved survival in patients with improved tumor vasculature and blood perfusion after treatment. This hypothesis-generating study supports the notion that excessively decreasing vascular permeability and pruning/rarefaction after bevacizumab therapy may negatively impact the outcome of combination therapy in NSCLC patients. This hypothesis warrants further dose-titration studies of bevacizumab to examine the dose effect on tumor vasculature and treatment efficacy.

lung cancer | antiangiogenesis | bioimaging

The advent of targeted therapies has led to an unprecedented increase in the median overall survival (OS) in advanced non–small cell lung cancer (NSCLC), well beyond 1 y. This progress included the successful development of antiangiogenic drugs such as bevacizumab or ramucirumab in combination with chemotherapy (1, 2). However, although the use of cancer cell-targeted drugs is guided by biomarkers (e.g., *EGFR* mutations, *ALK-EML4* translocations), there are currently no biomarkers for antiangiogenic drugs. In advanced NSCLC, the use of the anti-VEGF antibody bevacizumab in combination with platinum-based chemotherapy is a widely accepted standard of care in nonsquamous histology (1). However, the mechanism by which bevacizumab improves survival over chemotherapy alone remains debated.

Originally, it was hypothesized that antiangiogenic agents would effectively starve the tumor of oxygen and nutrients by pruning the blood vessel system and reducing blood perfusion to tumors (3). However, this effect would eventually lead to both decreased drug delivery (and hence treatment resistance) and increased tumor hypoxia (a major driver of tumor progression) (4). Another potential mechanism of antiangiogenic therapy is

Significance

A better mechanistic understanding of the survival benefits and identification of biomarkers of response would greatly enhance the optimal utilization of antiangiogenic agents such as bevacizumab in combination with chemotherapy in the treatment of cancer. This study indicates that the benefits of bevacizumab with chemotherapy in non–small cell lung cancer (NSCLC) patients may depend on tumor vascular function during treatment. These correlative studies also provide new insights into the selection of NSCLC patients most likely to benefit from the addition of bevacizumab treatment to chemotherapy. The imaging and circulating biomarker candidates should be further evaluated in larger studies.

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transient vascular normalization, by which structurally and functionally abnormal tumor vasculature is normalized, i.e., remodeled or modified to more closely resemble normal vasculature (4). By attenuation of vascular hyperpermeability, increasing vessel pericyte coverage, and normalization of the basement membrane, vascular normalization may lead to reduced interstitial fluid pressure and improved blood perfusion. This normalization may have important consequences on drug and oxygen delivery and on the immune microenvironment (5–8). Finally, it remains unclear whether bevacizumab's efficacy is dependent on the chemotherapeutic regimen used.

Unfortunately, clinical data exploring these mechanisms in NSCLC are scarce. Van der Veldt et al. reported that bevacizumab reduced perfusion and net influx rate of radiolabeled docetaxel in 10 patients with advanced NSCLC (9). Bevacizumab administration significantly reduced drug uptake measured by PET starting at 5 h after and persisting until day 4. Whether this effect was associated with benefit or treatment resistance remains unknown. Furthermore, although the carboplatin and albumin-bound paclitaxel (nab-paclitaxel) regimen has shown superiority in response rate over carboplatin and paclitaxel in a large phase III study (10) leading to its approval for NSCLC patients, the addition of bevacizumab to this regimen is yet to be thoroughly tested. We conducted a phase II trial that investigated the regimen of carboplatin and nab-paclitaxel in combination with bevacizumab for the first-line treatment of patients with advanced (stage IIIB/IV) nonsquamous NSCLC. In this study, we evaluated imaging and circulating biomarkers after bevacizumab alone and after bevacizumab plus chemotherapy to explore potential biomarkers of treatment response and resistance to this regimen. We found that markers of improved tumor blood perfusion after anti-VEGF therapy with carboplatin and nab-paclitaxel were associated with improved survival in these patients.

Results

Bevacizumab with Carboplatin and Nab-Paclitaxel Has Promising Antitumor Activity.
This study enrolled 36 patients between June 2008 to February 2012 at the Massachusetts General Hospital and Dana-Farber Cancer Institute. One patient experienced hemoptysis after enrollment and was taken off study before receiving any study drugs. The median age was 64, with 18 (50%) male patients. Table S1 describes the patient characteristics of the study population. Table 1 describes efficacy results in this population. Eight of the 36 patients (22%) were not evaluable for response, because they did not reach the planned first restaging CT scans scheduled after two cycles of combination chemotherapy. Of these eight patients, one patient was withdrawn before receiving any systemic treatment due to hemoptysis, as described above. One patient was taken off study after induction bevacizumab and before cycle 1 of combination chemotherapy as his epidermal growth factor receptor (EGFR) mutation status returned positive during that

Table 1. Efficacy of bevacizumab with nab-paclitaxel and carboplatin

Best response	n (%)
PR	13 (36%)
SD	13 (36%)
PD	2 (6%)
Not evaluable	8 (22%)
Rate	% (95% CI)
6-mo PFS	74 (57–97)
6-mo OS	79 (67–94)
Median	mo (95% CI)
Median PFS	8.5 (6.0–38.7)
Median OS	12.2 (9.6–44.1)

time, and the treating physician elected to take him off study to start an EGFR inhibitor. One patient came off study to receive palliative radiation to a painful bony metastasis, which was not detected before enrollment. The other five patients who were not evaluable stopped study treatment due to adverse events (AEs): one patient came off study after one cycle due to an allergic reaction; one patient came off study due to abscess/diverticulitis and a new finding of concurrent pancreatic cancer; two patients were taken off study as their liver function tests did not meet criteria to continue the study drug as written in the protocol and therefore required dose holds (although these were grade 2 liver function test abnormalities and were considered clinically acceptable to continue chemotherapy off study); and one patient came off study due to nausea/vomiting that was not tolerable. All of the 28 remaining patients were evaluable for response. Thirteen (36%) had a partial response (PR) and 13 (36%) had stable disease (SD) as their best response, and 2 (6%) showed progressive disease (PD). The primary end point of the study [6-mo progression-free survival (PFS) rate] was 74% (95% CI: 57, 97), and the 1-y PFS rate was 39% (95% CI: 21, 74). Median time to progression was 8.5 (95% CI: 6.0, 38.7) mo. The 6-mo OS rate was 79% (95% CI: 67, 94), and the median OS was 12.2 (95% CI: 9.6, 44.1) mo. OS and PFS distributions are shown in Fig. 1. AEs were generally manageable, and expected from this combination of therapies (Table S2). The most common grade 1–2 AEs included fatigue, nausea, alopecia, and anemia. The most common grade 3–4 AEs were fatigue, neutropenia, and thrombotic events (DVT/PE). Seven patients (19%) had a deep venous thrombosis (DVT) or pulmonary embolism (PE), which were all manageable with standard anticoagulation. Of note, two patients had incidentally noted PEs on restaging. Higher systolic blood pressure at day 1 of cycles 1 and 2 was associated with better OS and PFS.

Tumor Genotyping.
Tumor genotyping was available for 25 of 36 patients. There were 17 who were WT at all tested loci, 4 with KRAS mutations, 2 with an EGFR mutation, and 1 each with an ALK-EML4 gene rearrangement and ROS gene rearrangement. These numbers were too small to perform detailed analysis of genotype with biomarkers or outcome.

Treatment with Bevacizumab and Chemotherapy Decreases Tumor Blood Flow and Volume, P-S Product.
After the induction bevacizumab dose, perfusion CT measured blood flow (BF), blood volume (BV), and permeability-surface area (P-S) product were significantly decreased, and these effects were sustained throughout the combination therapy. Mean transit time (MTT) was not changed after bevacizumab alone or after two cycles of combination chemotherapy (at day 14 and cycle 3; Table S3). There was a significant and profound decrease in PET-measured standardized uptake value $(SUV)_{mean}$ and SUV_{max} after two and four cycles of combination therapy (Table S3).

The Extent of P-S Product Decrease and MTT Increase Inversely Associates with Survival.
When tested for associations with outcome, an increase in MTT during combination therapy, an index of decreasing perfusion, was inversely associated with OS ($P < 0.05$; Table 2). Interestingly, there was a nonsignificant trend for association between superior OS and the extent of P-S product increase after bevacizumab alone (HR = 0.87; $P = 0.052$) and a decrease after combination therapy (HR = 1.29; $P = 0.071$; Table 2). Next, we stratified patients by biomarker quartiles and found that Kaplan–Meier estimates of OS were increased in the groups with higher ΔP-S product (after bevacizumab alone) and lower ΔMTT (during combination treatment) (Fig. 2).

Of PET biomarkers, SUVmean and SUVmax at baseline—but not their change during treatment—were inversely associated with OS (Table 2). BV or BF at baseline or their changes after
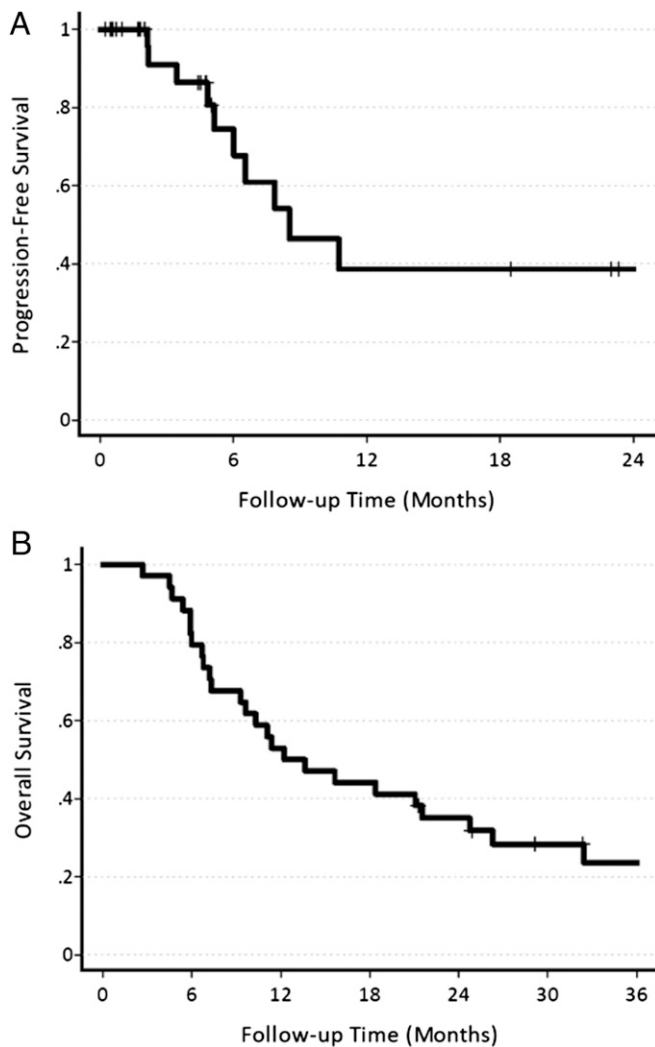
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Fig. 1. Survival outcomes after bevacizumab alone followed by combination of bevacizumab with chemotherapy in NSCLC patients. Kaplan–Meier distributions for PFS (*A*) and OS (*B*).

treatment at any time point did not associate with survival outcomes.

Circulating Biomarkers. We examined changes in angiogenic biomarkers and circulating blood cell populations both after the induction bevacizumab dose and while on combination therapy with bevacizumab with carboplatin and nab-paclitaxel and their associations with outcomes. Plasma placental-derived growth factor (PlGF) increased after bevacizumab induction and remained increased throughout therapy, whereas free plasma VEGF decreased after bevacizumab induction and remained decreased through therapy (Table S4). There was a transient decrease in plasma soluble (s)VEGFR1 measured at day 7 and a decrease in VEGF-C throughout therapy (Table S4). There were no significant changes on treatment in plasma SDF1α, bFGF, or sTie-2. Finally, bevacizumab alone decreased the number of CD34+CD133+ circulating progenitor cells (CPCs) at day 14 but did not affect the CD14+ circulating monocyte counts (Table S5). Exploratory studies showed that higher levels of plasma sVEGFR1 after bevacizumab induction (before combination therapy) associated with shorter OS (HR = 1.31; $P < 0.01$) and tended to associate with shorter PFS (HR = 1.58; $P = 0.054$; Table 3). Moreover, higher counts of circulating CD14+ monocytes at baseline and

after bevacizumab alone correlated with shorter OS and PFS ($P < 0.05$; Table 3). There was no significant correlation between other plasma or cellular biomarkers and survival outcomes.

Discussion

This open label phase II study was designed to investigate the combination of nab-paclitaxel with carboplatin and bevacizumab in the first-line treatment of advanced NSCLC. Nab-paclitaxel (Abraxane; Celgene) is a 130-nm albumin-bound formulation of paclitaxel, previously developed to improve the therapeutic index of paclitaxel. In preclinical models, nab-paclitaxel has demonstrated higher drug concentration in tumors compared with solvent-based paclitaxel (11). This regimen was therefore a logical combination to test in the first-line treatment of NSCLC, and this open label phase II trial was designed to investigate activity in this setting in combination with bevacizumab.

Bevacizumab with carboplatin and paclitaxel chemotherapy is a standard first-line treatment of nonsquamous NSCLC based on the Eastern Cooperative Oncology Group (ECOG) 4599 trial
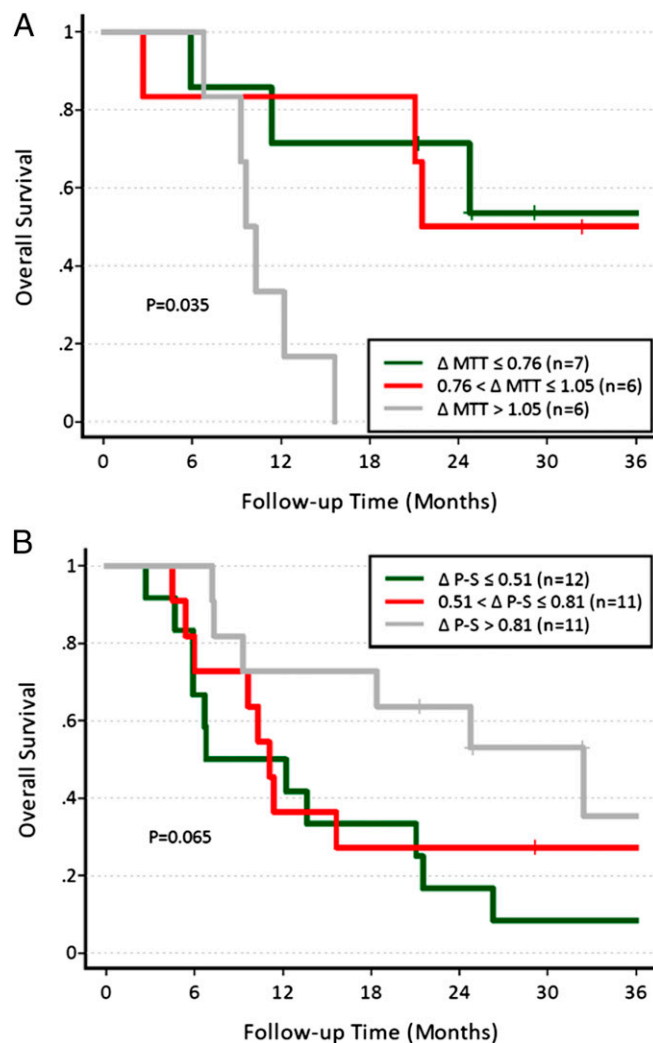
Fig. 2. Association between changes in functional vascular parameters after bevacizumab alone and after bevacizumab with chemotherapy and overall survival in NSCLC patients. (*A*) Kaplan–Meier OS distributions for changes in MTT during combination therapy. Note the favorable OS in the tertiles with low ΔMTT values. (*B*) Kaplan–Meier OS distributions for changes in P-S product after bevacizumab alone and before combination therapy. Note the poor OS in the tertiles with low ΔP-S values.

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Table 2. Correlations between baseline and on treatment imaging biomarker levels, OS, and PFS after bevacizumab alone followed by combination of bevacizumab with chemotherapy in NSCLC patients

Biomarker/time point	Pretreatment (baseline)		Change after bevacizumab alone (day 14)		Change after combination treatment			
					Before cycle 3		Before cycle 5	
	PFS	OS	PFS	OS	PFS	OS	PFS	OS
BF	1.29	1.00	0.98	0.95	0.80	0.95	1.12	0.92
	(0.98, 1.70)	(0.87, 1.15)	(0.82, 1.18)	(0.85, 1.07)	(0.60, 1.07)	(0.79, 1.14)	(0.72, 1.74)	(0.71, 1.19)
	$n = 34$	$n = 34$	n = 34	n = 34	$n = 19$	$n = 19$	$n = 10$	$n = 10$
P value	0.070	0.99	0.83	0.44	0.13	0.58	0.61	0.55
BV	1.23	0.99	0.90	0.96	0.88	1.03	1.02	1.25
	(0.95, 1.58)	(0.87, 1.14)	(0.74, 1.09)	(0.85, 1.09)	(0.68, 1.13)	(0.85, 1.25)	(0.69, 1.53)	(0.93, 1.68)
	$n = 34$	$n = 34$	$n = 34$	$n = 34$	$n = 19$	$n = 19$	n = 10	n = 10
P value	0.12	0.94	0.27	0.56	0.31	0.74	0.91	0.14
P-S	1.11	1.02	0.87	0.87	0.98	0.97	1.34	1.29
	(0.91, 1.36)	(0.91, 1.15)	(0.69, 1.09)	(0.76, 1.00)	(0.77, 1.25)	(0.81, 1.17)	(0.87, 2.06)	(0.98, 1.71)
	n = 34	n = 34	n = 34	n = 34	$n = 19$	$n = 19$	$n = 10$	$n = 10$
P value	0.29	0.73	0.21	0.052	0.88	0.78	0.18	0.071
MTT	0.95	0.96	0.99	1.03	1.11	1.25	1.23	1.29
	(0.78, 1.15)	(0.85, 1.09)	(0.83, 1.18)	(0.91, 1.15)	(0.87, 1.41)	(1.01, 1.55)	(0.78, 1.94)	(0.98, 1.71)
	$n = 34$	$n = 34$	$n = 34$	$n = 34$	$n = 19$	$n = 19$	$n = 10$	$n = 10$
P value	0.58	0.56	0.90	0.66	0.41	0.040	0.37	0.068
SUVmean	1.24	1.23	NA		1.04	1.00	1.01	1.01
	(0.94, 1.63)	(1.07, 1.41)			(0.85, 1.27)	(0.88, 1.15)	(0.84, 1.21)	(0.84, 1.23)
	$n = 30$	$n = 30$			$n = 27$	$n = 27$	$n = 18$	$n = 18$
P value	0.14	0.0042			0.70	0.95	0.91	0.88
SUVmax	1.21	1.23	NA		1.05	0.97	0.98	0.98
	(0.92, 1.59)	(1.07, 1.42)			(0.86, 1.27)	(0.85, 1.11)	(0.76, 1.25)	(0.82, 1.18)
	$n = 30$	$n = 30$			$n = 27$	$n = 27$	$n = 18$	$n = 18$
P value	0.17	0.0043			0.65	0.67	0.85	0.86

Data are shown as HRs from Cox regression using rank-transformed covariates. NA, not applicable.

(1). Efforts to improve on the outcomes have included combining bevacizumab with other agents, including carboplatin and pemetrexed (12), among others, but thus far, no regimen has shown clear superiority. In this single institution phase II study, the combination of bevacizumab with carboplatin and nab-paclitaxel was well tolerated, with a promising PR rate of 36% and a 6-mo PFS rate of 74%, with a median PFS of 8.5 mo and OS of 12.2 mo. These results are consistent with another single institution study of this combination, which reported a PR rate of 31% and a median PFS of 9.8 mo and OS of 16.8 mo (13). These data support further exploration of this regimen, but they also warrant a better understanding of the interaction between bevacizumab and chemotherapeutic agents in NSCLC. DVT or PE occurred in 19% of the patients, a higher rate than the 5–7% rates reported in phase III studies of bevacizumab. The reasons for this high rate are unclear, but it may reflect a slightly different patient population in our single Dana–Farber/Harvard Cancer Center (DF/HCC) institution study or increased detection due to the refinement of imaging techniques over time to detect incidental

thrombotic events. Interestingly, higher systemic blood pressure during combination therapy was significantly associated with longer PFS and OS. Hypertension is a class effect of anti-angiogenic therapy, and analyses across multiple tumor types have shown that hypertension is associated with better outcomes (14–17). These data support the notion that hypertension as an adverse event should be managed medically in an effort to continue bevacizumab therapy.

A key exploratory objective of our study was to use imaging and circulating biomarkers to identify potential mechanisms of action and biomarkers for response to this bevacizumab-based regimen. Identification of biomarkers of response would allow more rational use of bevacizumab in combination with chemotherapy agents and would fill an unmet need in the current utilization of angiogenic therapies. A previous study proposed that the mechanism of action of bevacizumab in NSCLC is reduction of chemotherapy uptake (9). In the current study, we did not directly measure the uptake of the chemotherapy agents into tumor. However, we measured the effect of bevacizumab on

Table 3. Correlations between baseline and day 14 circulating biomarker levels, OS, and PFS after bevacizumab alone followed by combination of bevacizumab with chemotherapy in NSCLC patients

Biomarker/time point	Pretreatment (baseline)		After bevacizumab alone (day 14)	
	PFS	OS	PFS	OS
sVEGFR1	0.92 (0.73, 1.17) $n = 34$	1.00 (0.88, 1.15) $n = 34$	1.58 (0.99, 2.52) $n = 22$	1.31 (1.09, 1.58) $n = 22$
P value	0.51	0.95	0.054	0.0038
Circulating CD14+ monocyte counts	1.29 (1.01, 1.65) $n = 31$	1.23 (1.06, 1.43) $n = 31$	1.07 (0.75, 1.52) $n = 18$	1.30 (1.06, 1.60) $n = 18$
P value	0.045	0.0063	0.72	0.013

Data are shown as HRs from Cox regression using rank-transformed covariates.

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tumor BF, BV, P-S product, and MTT (a measure of blood perfusion), with the goal of exploring the mechanism of survival benefit. In line with published data in NSCLC and other cancers, we found that bevacizumab significantly decreased median BF, BV, and P-S product at all time points analyzed in this NSCLC patient population. The change in MTT was more heterogeneous between patients. These imaging biomarker data indicate that, as seen in other cancers, bevacizumab can reduce both vascular permeability and surface area and glucose uptake. However, this effect did not associate with a survival benefit. On the contrary, the extent of the decrease in P-S product after bevacizumab alone tended to associate with shorter OS, whereas the change in BF, BV, or MTT at this time point showed no association with survival outcomes. In line with this finding, during combination treatment, the increase in MTT (i.e., a reflection of decreased blood perfusion) associated with shorter OS. Further prospective studies of changes in P-S product and MTT as potential imaging biomarkers of response are warranted.

Although exploratory in nature, these findings support the notion that excessive vascular pruning/rarefaction and decreased permeability after bevacizumab may in fact negatively impact the outcome of combination therapy in NSCLC patients. These hypothesis-generating data suggest that the ability of bevacizumab to induce a more normalized, functional vasculature, and maintain appropriate tumor perfusion in a subset of patients may be a determinant of response to cytotoxic therapy and ultimately for increased OS. It may be worthwhile to consider testing different doses of bevacizumab in the future to further examine the dose effect on perfusion and permeability especially in combination with different chemotherapies (4). The feasibility and efficacy of a lower dose of bevacizumab in NSCLC patients has been demonstrated (18). Moreover, preclinical studies have demonstrated that lower doses of antiangiogenics may lead to improved drug distribution and reprogramming of the immune microenvironment toward an antitumor phenotype (19, 20).

Consistent with multiple prior studies of anti-VEGF/VEGFR agents, including bevacizumab in sarcoma, rectal, breast, and ovarian cancer, we found that anti-VEGF therapy led to a sustained increase in PlGF, suggesting that this may be a pharmacodynamic marker of activity (21–28). We also found that, although pretreatment plasma VEGF does not associate with outcome, higher levels of sVEGFR1 before combination therapy were associated with worse survival. Multiple previous studies have shown that high levels of sVEGFR1 are correlated with worse clinical outcomes, making sVEGFR1 a potential biomarker of intrinsic resistance to antiangiogenic therapy (22, 28). Finally, the number of circulating CD14+ monocytes, a potential biomarker of systemic inflammation, associated with poor survival. These results are in line with the preclinical data suggesting that monocyte/macrophages may contribute to resistance to antiangiogenic therapy (4). These potential biomarkers should be further examined in larger randomized studies.

In summary, bevacizumab with nab-paclitaxel and carboplatin has promising activity in the first-line treatment of advanced NSCLC. Exploratory imaging studies showed that overall, the bevacizumab-based treatment reduced blood flow, volume, and permeability. However, they also showed that survival benefits might be related to improved rather than impaired tumor perfusion in these patients.

Methods

Patients. Patients with histologically confirmed NSCLC were enrolled in a prospective phase II clinical trial of bevacizumab in combination with carboplatin and nab-paclitaxel (NCT00642759). This study was approved by the DF/HCC Institutional Review Board and all patients signed informed consent. Eligible patients had nonsquamous histology, stage IIIB (by pleural or pericardial effusion) or IV by the American Joint Committee on Cancer (AJCC) sixth

edition criteria, ECOG performance status of 0–1, and no prior chemotherapy for their advanced disease.

Treatment and Study Design. Patients were treated with a single dose of i.v. bevacizumab 15 mg/kg as induction therapy. After 14 d, on cycle 1, day 1, patients started combination therapy with i.v. carboplatin, nab-paclitaxel, and bevacizumab, with carboplatin given at a target area under the curve of 6 using the Cockroft–Gault equation (29) on day 1, nab-paclitaxel 100 mg/m^2 on days 1, 8, and 15, and bevacizumab 15 mg/kg on day 1 of every 21-d cycle. After completion of a maximum of six cycles of combination therapy, patients were allowed to continue with maintenance bevacizumab 15 mg/kg every 21 d.

Tumor Genotyping. When available, tumors were tested for molecular alterations using the SNaPshot genotyping platform (30), a validated, Clinical Laboratory Improvement Amendments (CLIA)-approved multiplexed tumor genotyping assay that is used for real-time testing of tumors. This assay uses formalin-fixed paraffin-embedded tissue to quickly and economically identify 58 commonly mutated loci in 14 key oncogenes. FISH testing was used to check for ALK and ROS translocations.

Functional Imaging. Correlative studies included perfusion CT scans at baseline, after induction bevacizumab (and before combination chemotherapy), and before cycles 3 and 5 of combination therapy (Fig. S1). Perfusion CT was performed on a 16/64-slice multidetector row CT scanner (Light Speed/Discovery; GE Medical Systems). A dynamic CT scan of this region was performed for 45 s at the same table position 5–8 s after the start of i.v. injection of 50 mL of iodinated nonionic contrast media (Isovue 370; Bracco Diagnostics) at a rate of 7 mL/s. Subsequently, delayed phase images of the tumor were obtained every 14 s for about 3 min. CT-perfusion data were analyzed on a workstation (Advantage Windows; GE Medical Systems) using commercially available software (CT Perfusion 3.0; GE Medical Systems). We evaluated changes over time in BF, BV, P-S product, and MTT (a negative measure of tumor blood perfusion). The dynamic CT scan for perfusion imaging was limited to 2–4 cm of chest anatomy in the z axis. A clearly defined target tumor measuring >1 cm in diameter in the lung or mediastinum was considered for perfusion analysis. Regions of interest were manually drawn to include the entire tumor on all of the slices. PET scanning was performed on a PET-CT scanner (Biograph 64; Siemens Medical Solutions), and images were acquired 1 h after i.v. injection of 15 mCi (555 MBq) of 18F-FDG. PET data were analyzed on a Syngo TrueD workstation (Siemens). SUV$_{mean}$ and SUV$_{max}$ (31) using 18FDG-PET were also obtained at baseline and before cycles 3 and 5 of combination therapy. In exploratory studies, we evaluated the association of baseline and on-treatment changes with PFS and OS.

Circulating Biomarkers. Correlative studies also included measurement of circulating molecules and cells at baseline, at days 7 and 14 after bevacizumab alone (before combination chemotherapy), and before cycles 3 and 5 of combination therapy (Fig. S1). To this end, peripheral blood was obtained from all patients enrolled. Plasma samples were separated by centrifugation and then aliquoted and stored at –80 °C until they were used for ELISA measurements of free (non–bevacizumab-bound) VEGF, PlGF, sVEGFR1, basic fibroblast growth factor (bFGF), VEGF-C, VEGF-D, and sTie2 using a CLIA-certified multiplex (7-plex) protein array from Meso-Scale Discovery, and stromal-derived factor 1α (SDF1α) using ELISA kits from R&D Systems. All samples were run in duplicate. The number of CD133+CD34+CD45dim CPCs and CD14+CD45 + monocytes were counted by flow cytometry using a LSR-II cytometer and FACSDiva software (BD).

Statistical Analysis. The primary end point of this study was to determine the 6-mo PFS rate in the study population. Secondary end points were the safety and tolerability of the combination regimen, the overall response rate, and overall survival to the combination regimen. Exploratory end points were perfusion imaging and circulating biomarker correlations with response. PFS was counted from enrollment until death or tumor progression assessed using RECIST version 1.0 (32). With the planned sample size of 36 subjects, the study was designed to have 80% power of detecting the difference in 6-mo PFS rates between 0.3 and 0.5, with a type I error of 0.047. Efficacy evaluations included all subjects who took at least one dose of the experimental regimen of carboplatin, nab-paclitaxel, and bevacizumab. Patients were censored on withdrawal from study treatment in the analysis of PFS and on the last follow-up in the analysis of OS. For survival end points, we report product-limit estimates with Peto's confidence intervals (33). All biomarker levels (plasma proteins, circulating cells, blood pressure

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measurements, functional imaging measurements) and their changes were described using quartiles; the changes in biomarkers were defined using ratios and assessed with the one-sample, two-sided, exact Wilcoxon test. The false discovery control method of Genovese et al. (34), with weights proportional to the square root of the number of paired measurements, was used to account for multiple comparisons over time; no adjustment was performed to account for multiple biomarkers because each was of interest in itself. Missing measurements of biomarkers were excluded from the analysis. The correlation of biomarkers with survival end points was analyzed using Cox regression with rank-transformed measurements or tertile-transformed (for functional imaging) measurements; we used

P values from the two-sided Wald test. $P < 0.05$ was considered statistically significant. The data were analyzed using R (R Foundation for Statistical Computing).

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