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ORIGINAL REPORT

Distinct Patterns of Cytokine and Angiogenic Factor Modulation and Markers of Benefit for Vandetanib and/or Chemotherapy in Patients With Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
There is an unmet need for biomarkers for identifying patients likely to benefit from anticancer treatments, selecting dose, and understanding mechanisms of resistance. Plasma vascular endothelial growth factor (VEGF) and soluble VEGF receptor 2 (sVEGFR-2) are known to be modulated by VEGF pathway inhibitors. It is unknown whether chemotherapy or VEGFR inhibitor/chemotherapy combinations induce changes in these or other cytokines and angiogenic factors (CAFs) and whether such changes could be markers of benefit.

Methods
Thirty-five plasma CAFs were analyzed using multiplexed bead arrays and enzyme-linked immunosorbent assays from 123 patients with non–small-cell lung cancer in a randomized phase II study who received vandetanib, a VEGFR and epidermal growth factor receptor inhibitor, monotherapy carboplatin and paclitaxel (CP), or the combination (VCP). Changes in CAFs at days 8, 22, and 43 from baseline were correlated with progression risk.

Results
VEGF increased and sVEGFR-2 decreased by day 43 in the vandetanib arm, whereas a distinct pattern was observed in the CP and VCP arms, with significant decreases in interleukin (IL) -12, IL-1 receptor antagonist, and matrix metalloproteinase 9 (MMP-9) and increased macrophage chemoattractant protein 1. In each treatment arm, changes in different markers were associated with progression risk. For example, increases in IL-8 with VCP, MMP-9 with CP, and VEGF with vandetanib monotherapy were associated with increased progression risk, and increase in intercellular adhesion molecule 1 with vandetanib was associated with decreased risk.

Conclusion
Vandetanib and chemotherapy treatment led to distinct patterns of CAF changes; the combination resembled chemotherapy alone. Changes in specific CAFs correlated with clinical outcome, but markers differed for each treatment arm. CAF profiling may provide insights into the biologic effects of treatment and identify drug-specific markers of activity and clinical benefit.

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INTRODUCTION

Angiogenesis is an essential process for tumor growth and metastatic spread.[1,2] The balance of proangiogenic and antiangiogenic factors, including growth factors, cytokines, and chemokines, that regulate physiologic angiogenesis is disrupted during tumorigenesis.[3-5] Vascular endothelial growth factor (VEGF) is a critical proangiogenic factor that is upregulated in tumors.[4] Inhibitors of VEGF signaling, including bevacizumab, sorafenib, and sunitinib, have proven clinical benefit for the treatment of several solid tumors, and many similar agents are in development.[6-13]

However, clinical trials using such molecularly targeted therapies present some problems that do not typically occur in trials of cytotoxic agents. The optimal antitumor effect of these agents may occur at doses below the clinically defined maximum-tolerated dose. This has made determination of the recommended dose for phase II and III testing difficult, as demonstrated by the various doses

of bevacizumab used in pivotal phase III trials.[6-9,14] Furthermore, antiangiogenic agents may be cytostatic, rather than cytotoxic, which has made determination of their clinical efficacy and optimal dosing challenging.

Clinical evaluation and use of antiangiogenic agents would be greatly facilitated by the identification of biomarkers that are modulated by the therapies. Such modulated biomarkers could have the potential to be used as activity biomarkers to determine the optimal antitumor dose,[15] to predict clinical benefit early in the course of therapy, to monitor responses to treatment, and to enhance our understanding of the mechanisms of action of and resistance to therapeutic agents.

Increases in VEGF and decreases in soluble VEGF receptor 2 (sVEGFR-2) have been commonly reported in phase I and II studies of VEGFR tyrosine kinase inhibitors (TKIs) and seem to be a class effect of these agents.[16-19] However, only some studies have found associations between these factor changes and clinical benefit.[16,18-22] Recently, Ebos et al[16] showed that these VEGF and VEGFR-2 changes in tumor-bearing and non–tumor-bearing mice treated with sunitinib (VEGFR/platelet-derived growth factor receptor/c-kit inhibitor) occur as a result of a systemic, tumor-independent response that is dose dependent and coincides with the predetermined optimal antitumor dose of sunitinib. The impact of VEGFR TKIs and other therapeutic agents, such as chemotherapy, on the broader profile of cytokines and angiogenic factors (CAFs) in cancer patients is not well understood. Recent preclinical studies suggest that such changes may be biologically important.[23]

Vandetanib is an orally administered TKI of VEGFR-2, epidermal growth factor receptor (EGFR), and RET that, as monotherapy or in combination with chemotherapy, has improved progression-free survival (PFS) in patients with advanced non–small-cell lung cancer (NSCLC) in three phase II studies and is now being evaluated in phase III settings.[24-26] We performed serial assessments of plasma levels of 35 CAFs, including VEGF and sVEGFR-2, among the patients in a randomized phase II trial who were randomly assigned to vandetanib monotherapy, carboplatin and paclitaxel (CP) chemotherapy, or vandetanib in combination with CP (VCP) for the first-line treatment of advanced NSCLC. In this trial, VCP demonstrated a PFS benefit compared with CP, but vandetanib was inferior to CP.[24] The three-arm design of this study provided the unique opportunity to identify patterns of changes in CAF concentrations over time during therapy with vandetanib, CP chemotherapy, and the VCP combination and to correlate these changes with progression risk.

METHODS

Patients and Study Design

The multicenter, randomized, phase II clinical trial is described in detail elsewhere.[24] Patients with chemotherapy-naïve stage IIIB or IV NSCLC (N = 181) were assigned (2:1:1) to vandetanib 300 mg by mouth once daily until disease progression or intolerance, CP (carboplatin area under the curve 6; paclitaxel 200 mg/m^2) intravenously once every 3 weeks for six cycles, or same-dose CP for six cycles in combination with vandetanib 300 mg/d until progression or intolerance. The primary objectives were to determine whether vandetanib monotherapy was noninferior to CP and whether VCP prolonged PFS compared with CP. This clinical study was approved by all relevant institutional ethical committees or review bodies and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Each patient provided written informed consent. Consent for plasma sample collection for biomarker analyses was optional, and only patients who consented are included in this analysis.

Plasma Sample Collection and Analyses

Plasma samples were prepared from venous blood samples collected at baseline (day −7 to pretreatment on day 1), day 8 (D8; ± 1 day), day 22 (D22; ± 3 days), and day 43 (D43; ± 3 days), frozen, and stored at −70 to

Table 1. Cytokines and Angiogenic Factors Analyzed

Factor Analyzed
Proangiogenic factors
VEGF
bFGF
EGF
TNF-α
IL-6
IL-8
IL-1b
MMP-9
HGF
MCP-1
Antiangiogenic factors
IL-12p40/70
INF-α
INF-γ
MIG
IP-10
Inflammatory markers
ICAM-1
Markers of hypoxia
Osteopontin
Hematopoietic growth factors
GM-CSF
G-CSF
Markers of endothelial cell function or damage
E-selectin
sVEGFR-2
Other interleukins
IL-1RA
IL-2
sIL-2R
IL-4
IL-5
IL-7
IL-10
IL-13
IL-15
IL-17
Other cytokines and chemokines
RANTES
MIP-1α
MIP-1β
Eotaxin

Abbreviations: VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; TNF, tumor necrosis factor; IL, interleukin; MMP, matrix metalloproteinase; HGF, hepatocyte growth factor; MCP-1, macrophage chemoattractant protein-1; INF, interferon; MIG, monokine induced by interferon gamma; IP-10, interferon gamma–induced protein 10; ICAM-1, intercellular adhesion molecule 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; sVEGFR-2, soluble vascular endothelial growth factor receptor 2; IL-1RA, interleukin-1 receptor antagonist; sIL-2R, soluble interleukin-2 receptor; MIP, macrophage inflammatory protein.

−80°C until analysis (further handling details are in the Appendix, online only). We analyzed the plasma samples blinded to clinical outcome. Plasma concentrations of 35 CAFs (Table 1) were measured at each of the four time points. Thirty-three factors were analyzed with commercially available multiplexed bead suspension arrays, and osteopontin and sVEGFR-2 were analyzed by enzyme-linked immunosorbent assays (product/manufacturer details can be found in the Appendix), all per the manufacturers' instructions. Each sample was analyzed in duplicate. CAF concentrations from all time points for each patient were analyzed in the same enzyme-linked immunosorbent assays and multiplexed bead suspension arrays to minimize interexperimental variability.

Statistical Methods

The patient demographics and disease characteristics of the clinical study patients with and without CAF data were compared using the χ^2 test for categoric variables and the Wilcoxon rank sum test for continuous variables. Linear mixed models were used to study the marker changes over time.[27] The transformation of logarithm to the base 2 of a marker was used in this analysis to satisfy the normality assumption. Treatment × time interaction on marker levels was assessed, and subgroup analyses by treatment arm were carried out. Regression analyses using the Cox proportional hazards model were conducted on PFS. The change over time of a CAF compared with baseline was calculated as the difference of CAF concentration at each time point from concentration at baseline on the log-transformed scale. We tested the interaction between the change over time of each CAF and treatment first. Subgroup analyses were performed to further test the effect of CAF change on PFS within each treatment group in the Cox model. Similar results were obtained with and without adjusting for sex and smoking status (smoker *v* nonsmoker), which were found to be significant baseline prognostic and/or predictive factors; adjusted results are presented here. All *P* values are two-sided. We considered $P < .05$ to be significant. We did not control for multiple analyses because these analyses are exploratory. SAS Version 9.1 (SAS Institute, Cary, NC) and S-Plus Version 7.0 (Statistical Sciences, Seattle, WA) were used to carry out the computations for all analyses.

RESULTS

Patient Population

Baseline plasma samples were available from 123 (68%) of 181 patients in the trial (55 from the vandetanib arm, 32 from the CP arm, and 36 from the VCP arm). The characteristics of patients with baseline plasma samples and the number of samples analyzed per treatment arm and time point are listed in Table 2. The characteristics of these 123 patients did not differ significantly from those of the 58 patients without plasma available for analysis (data not shown). D8, D22, and D43 plasma samples were available from 104, 94, and 80 patients, respectively.

Table 2. Patient and Disease Characteristics

Characteristic	No. of Patients (N = 123)	%
Median age, years	61	
Sex		
Male	86	70
Female	37	30
Histology		
Adenocarcinoma	56	46
Squamous	28	23
Large cell	17	14
Adenocarcinoma with BAC features/BAC	5	4
Other	17	14
Stage		
IIIB	18	15
IV	105	85
Race		
White	112	91
Black	3	2
Asian	2	1.5
Other	6	5
Smoking status		
Current	31	25
Former	66	54
Never	25	20
Unknown	1	< 1
V arm, No. of plasma samples		
Baseline	55	45
Day 8	45	
Day 22	35	
Day 43	31	
CP arm, No. of plasma samples		
Baseline	32	26
Day 8	27	
Day 22	30	
Day 43	23	
VCP arm, No. of plasma samples		
Baseline	36	29
Day 8	32	
Day 22	29	
Day 43	26	

Abbreviations: BAC, bronchioloalveolar carcinoma; V, vandetanib; CP, carboplatin and paclitaxel; VCP, vandetanib, carboplatin, and paclitaxel.

CAF Changes During Vandetanib, Chemotherapy, and Combination Treatment

When patients from all three treatment arms were considered together, significant changes in the plasma concentrations of interleukin (IL) -12, IL-1 receptor antagonist (IL-1RA), IL-8, macrophage chemoattractant protein 1 (MCP-1), matrix metalloproteinase 9 (MMP-9), and sVEGFR-2 over time were detected. IL-12, IL-1RA, MMP-9, and sVEGFR-2 concentrations were lower at D8 compared with baseline ($P < .001$, $P = .003$, $P < .001$, and $P = .001$, respectively). IL-8 and MCP-1 concentrations were higher at D8 than at baseline ($P = .09$ and $P < .001$, respectively). Changes in basic fibroblast growth factor (bFGF), IL-12, and interferon-inducible protein 10 (IP-10) over time differed by treatment arm, with $P = .035$, .0021, and .023, respectively, for the interactions between treatment and these factor changes. There were also interactions of borderline significance between treatment and changes in IL-1RA and VEGF ($P < .10$).

We then performed subgroup analyses to assess per treatment arm for significant changes in each marker from baseline to each of the time points (D8, D22, and D43). The changes in CAF concentrations over time are reported here and are illustrated in Table 3, Figure 1, and Appendix Figure 1 (online only).

In the vandetanib monotherapy arm, plasma concentrations of VEGF significantly increased ($P = .048$) and concentrations of sVEGFR-2 significantly decreased ($P < .001$) from baseline to D43. There were also significant increases in IL-8 ($P = .041$) at D8 and in granulocyte colony-stimulating factor ($P = .03$) and IL-17 at D43 ($P = .045$). There were no significant CAF changes at D22 from baseline.

In the CP arm, there were significant decreases from baseline to D8 in plasma concentrations of IL-12 ($P < .001$), IL-1RA ($P = .009$),

Table 3. Changes in Plasma Concentrations of Cytokines and Angiogenic Factors During Treatment

CAF and Treatment Arm	Baseline			Day 8				Day 22				Day 43			
	No. of Patients	Median (pg/mL)	Range (pg/mL)	No. of Patients	Median (pg/mL)	Range (pg/mL)	% Baseline*	No. of Patients	Median (pg/mL)	Range (pg/mL)	% Baseline*	No. of Patients	Median (pg/mL)	Range (pg/mL)	% Baseline*
VEGF															
V	55	102	0-1,246	45	105	0-1,160	98	35	97	0-880	105	31	128	0-289	138†
CP	32	121	34-1,087	27	127	0-546	84	30	119	24-1,397	100	23	114	40-552	105
VCP	36	118	0-4,024	32	140	16-3,606	101	29	124	16-3,744	93	26	117	6-1,041	111
sVEGFR-2															
V	54	9,593	4,100-16,732	45	8,443	2,257-21,598	93	34	8,414	3,429-14,483	90	29	7,290	3,429-14,483	85†
CP	29	8,611	5,543-17,764	27	9,153	249-13,431	86†	28	8,877	3,110-15,457	95	22	9,097	939-16,897	92
VCP	34	9,201	2,953-17,594	30	8,529	1,294-17,673	99	29	8,994	3,895-15,995	90	25	8,638	1,111-20,221	97
IL-12															
V	55	219	38-1,230	45	211	54-1,292	97	35	276	44-1,178	97	31	258	54-1,406	88
CP	32	193	53-1,424	27	110	38-1,690	55†	30	199	37-1,774	91	23	193	58-1,380	79
VCP	36	218	46-1,543	32	124	37-1,014	59†	29	192	37-858	79	26	198	32-785	84
IL-1RA															
V	55	610	150-6,234	45	599	175-11,929	101	35	750	148-8,626	101	31	643	197-8,121	104
CP	32	493	188-27,612	27	338	88-18,191	66†	30	431	108-11,340	83	23	594	145-11,139	85
VCP	36	600	130-11,201	32	347	102-11,005	71†	29	507	109-10,351	80	26	538	95-7,516	74
MMP-9															
V	55	38,592	5,742-892,964	45	35,458	2,749-980,916	106	35	39,045	5,750-211,208	123	31	32,037	2,752-402,460	108
CP	32	45,165	6,305-2,500,000	27	24,574	1,771-188,733	51†	30	28,842	2,606-553,724	86	23	30,811	5,443-201,182	75
VCP	36	30,703	6,198-393,092	32	25,560	2,119-122,638	80†	29	41,787	9,489-174,531	128	26	28,625	2,587-196,677	106
MCP-1															
V	55	349	132-2,841	45	370	117-7,539	109	35	416	141-3,270	102	31	370	133-4,743	126
CP	32	358	140-4,587	27	576	147-3,161	136†	30	375	66-2,363	96	23	386	114-2,243	103
VCP	36	387	41-5,226	32	458	221-5,732	139†	29	451	72-5,076	102	26	485	143-4,925	116
Eotaxin															
V	55	33	8-150	45	33	11-235	92	35	32	13-224	95	31	35	13-178	102
CP	32	43	9-179	27	39	12-108	100	30	39	13-116	98	23	44	17-190	110
VCP	36	43	7-108	32	45	10-102	110	29	47	7-114	129	26	53	14-121	121†
G-CSF															
V	55	94	1-1,733	45	77	1-3,393	100	35	79	1-825	111	31	101	6-564	116†
CP	32	129	4-1,717	27	102	8-550	94	30	99	1-1,832	109	23	134	1-1,625	115
VCP	36	92	1-3,792	32	95	1-3,252	110	29	92	1-3,511	91	26	104	1-2,991	101
IL-4															
V	55	24	0-750	45	24	0-727	100	35	22	0-368	109	31	31	0-209	100
CP	32	23	0-217	27	17	0-267	79†	30	22	0-276	106	23	24	0-727	102
VCP	36	19	0-652	32	20	0-798	99	29	26	0-491	109	26	25	0-579	105
IL-8															
V	55	41	2-2,644	45	49	2-417	115†	35	41	6-219	131	31	38	11-238	123
CP	32	43	11-2,300	27	58	10-866	92	30	33	2-929	68	23	35	7-136	99
VCP	36	53	4-711	32	59	12-362	118	29	35	7-213	80	26	40	9-279	104
IL-10															
V	55	86	1-2,273	45	68	1-1,669	101	35	71	6-924	111	31	63	2-1,400	117
CP	32	80	15-6,176	27	64	10-2,044	63†	30	68	13-36,940	94	23	64	11-36,940	93
VCP	36	50	9-36,940	32	54	7-36,940	106	29	61	6-36,940	102	26	52	13-6,870	123
IL-13															
V	55	83	2-1,401	45	90	2-1,542	100	35	103	2-750	100	31	90	2-382	107
CP	32	95	2-1,598	27	78	2-926	81†	30	82	2-2,676	100	23	98	15-1,791	100
VCP	36	82	2-2,163	32	80	2-2,701	98	29	74	2-2,229	100	26	67	2-2,151	100
IL-17															
V	55	95	0-2,483	45	78	0-2,694	100	35	63	0-2,400	100	31	104	0-1,548	128†
CP	32	93	0-1,675	27	64	0-710	100	30	48	0-1,742	102	23	116	0-1,975	100
VCP	36	75	0-2,028	32	69	0-2,439	100	29	57	0-1,393	94	26	28	0-1,959	100
IP-10															
V	55	35	5-328	45	45	6-151	81	35	53	9-264	118	31	62	5-505	147
CP	32	36	9-889	27	30	12-265	105	30	30	6-106	96†	23	23	8-102	60†
VCP	36	33	6-6,044	32	33	12-530	110	29	33	3-1,609	94	26	43	3-1,388	88
MIP-1α															
V	55	70	9-1,786	45	70	2-4,016	94	35	62	3-2,945	102	31	56	2-2,149	99
CP	32	55	20-3,376	27	60	2-2,101	89†	30	53	17-1,427	102	23	77	19-1,830	101
VCP	36	53	2-1,471	32	41	9-1,421	90	29	51	2-1,112	84	26	58	2-1,014	90

NOTE. The data presented in this table are based on the raw data prior to $\log_2$ transformation.

Abbreviations: CAF, cytokine and angiogenic factor; VEGF, vascular endothelial growth factor; V, vandetanib; CP, carboplatin and paclitaxel; VCP, vandetanib, carboplatin, and paclitaxel; sVEGFR-2, soluble vascular endothelial growth factor receptor 2; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; MMP-9, matrix metalloproteinase 9; MCP-1, macrophage chemoattractant protein 1; G-CSF, granulocyte colony-stimulating factor; IP-10, interferon gamma–induced protein 10; MIP-1α, macrophage inflammatory protein 1α.

*Percent baseline indicates the median of the ratios of CAF concentration at each time point to baseline concentration expressed as a percentage (not the ratio of the median concentration at each time point to the median baseline concentration).

†In the Cox proportional hazards model using $\log_2$ transformation of data, $P < .05$ for change in CAF concentration from baseline to time point.

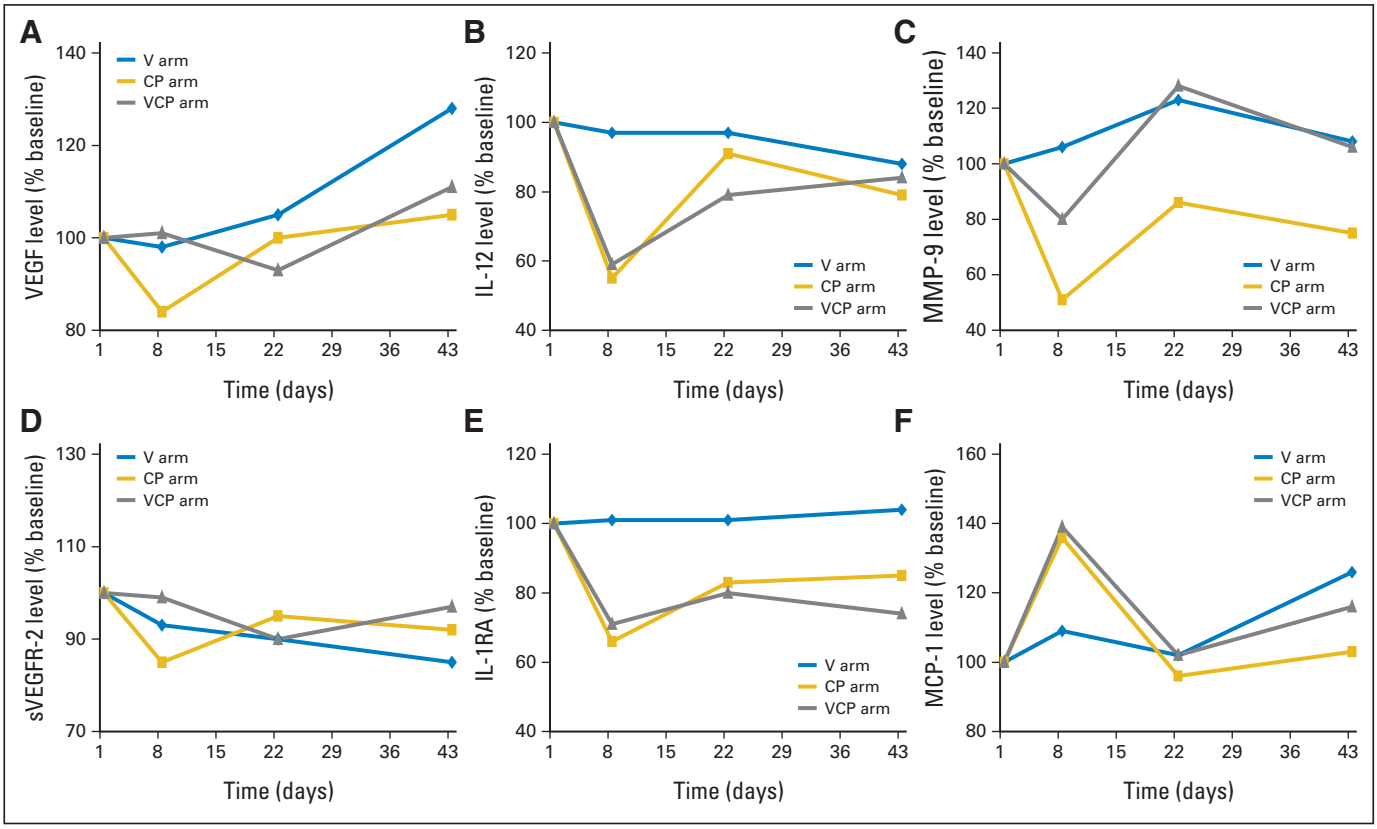

Fig 1. (A-F) Changes in concentrations of cytokines and angiogenic factors (CAFs) during treatment (% baseline indicates the median of the ratios of CAF concentration at each time point to baseline concentration expressed as a percentage, not the ratio of the median concentration at each time point to the median baseline concentration). These figures are based on raw data. The *P* values in the text are based on the analysis of $\log_2$ transformed data in mixed linear models and adjusted for sex. For figures generated from the $\log_2$ transformed data with error bars, please see Figure A1. CP, carboplatin and paclitaxel; VCP, vandetanib, carboplatin, and paclitaxel; VEGF, vascular endothelial growth factor; IL-12, interleukin-12; MMP-9, matrix metalloproteinase-9; sVEGFR-2, soluble vascular endothelial growth factor receptor 2; IL-1RA, interleukin-1 receptor antagonist; MCP-1, macrophage chemoattractant protein 1.

and MMP-9 ($P < .001$) and a significant increase in MCP-1 at D8 ($P = .013$). Other significant CAF changes in the CP arm were decreases in IL-4 ($P = .031$), IL-10 ($P = .043$), IL-13 ($P = .011$), sVEGFR-2 ($P = .024$), and macrophage inflammatory protein 1α ($P = .027$) at D8 and a decrease in IP-10 at D22 ($P = .036$) and D43 ($P = .012$).

In the VCP arm, there were similarly significant decreases from baseline to D8 in IL-12 ($P < .001$), IL-1RA ($P = .003$), and MMP-9 ($P = .035$) concentrations. There were significant increases in MCP-1 ($P = .004$) at D8 and eotaxin ($P = .016$) at D43. No significant CAF changes were detected at D22.

It is notable that even though vandetanib targets the VEGFR and EGFR pathways, no significant changes in epidermal growth factor levels over time were observed in any of the treatment arms.

Correlation Between CAF Changes and PFS

We found correlations between the changes in 14 CAF concentrations during treatment and PFS for individual treatment arms (Table 4). We then further tested whether the correlation between the change in a CAF and PFS differed between the treatment arms; that is, we assessed for interactions between treatment and the change-in-CAF concentration as a continuous variable. Six CAFs had significant interactions (Table 4). We further evaluated these CAFs by comparing patients with CAF changes ≤ or greater than (>) the median degree of change (Fig 2). For example, patients with a greater than median increase in intercellular adhesion molecule 1 (ICAM-1) concentration at D8 had a significantly improved PFS in the vandetanib arm and VCP arm compared with patients with a ≤ median increase, but there were no significant differences in outcome in the CP arm (P for interaction = .021; Fig 2A). Patients with a greater than median increase in VEGF levels had an inferior PFS in the vandetanib arm compared with patients with a ≤ median increase, but there were no significant differences in outcome in the CP or VCP arms (P for interaction = .009; Fig 2B).

DISCUSSION

In this exploratory analysis of plasma levels of 35 CAFs during treatment with vandetanib and/or CP chemotherapy for advanced NSCLC, we found that vandetanib and chemotherapy were associated with distinct patterns of CAF changes and that the CAF changes with the VCP combination resembled those with chemotherapy alone. In addition, the changes in specific CAFs that correlated with clinical outcome differed for each treatment arm. Our results are summarized in appendix Table A1 (online only). Interestingly, most of the significant associations between outcome and changes in CAF levels in our study were seen at D8, suggesting that these changes in markers could

Table 4. Associations Between Change in CAF Plasma Concentrations and PFS

Time Point and CAF	HR*	95% CI
Day 8		
VCP		
IL-8	1.48	1.02 to 2.16
IL-12	0.61	0.40 to 0.94
IP-10	0.69	0.48 to 0.98
MIP-1α	0.61	0.39 to 0.98
CP		
IL-13	0.70	0.55 to 0.89
IL-15	0.76	0.60 to 0.98
IL-17†	0.75	0.60 to 0.95
V		
ICAM-1†	0.53	0.30 to 0.96
VEGF†	1.93	1.06 to 3.52
Osteopontin	0.88	0.78 to 1.00
Day 22		
CP		
MMP-9	1.31	1.04 to 1.64
Day 43		
VCP		
IL-8†‡	1.56	0.98 to 2.48
IFN-α†‡	1.27	0.98 to 1.60
CP		
IL-15	1.74	1.04 to 2.90
V		
sIL-2R†	0.67	0.45 to 0.99
IL-5	0.83	0.71 to 0.96

NOTE. HR < 1.0 indicates that increase in CAF level correlates with improved PFS; HR >1.0 = rise in CAF level correlates with worse PFS.

Abbreviations: CAF, cytokine and angiogenic factor; PFS, progression-free survival; HR, hazard ratio; VCP, vandetanib, carboplatin, and paclitaxel; IL, interleukin; IP-10, interferon gamma–induced protein 10; MIP-1α, macrophage inflammatory protein 1α; CP, carboplatin and paclitaxel; V, vandetanib; ICAM-1, intercellular adhesion molecule 1; VEGF, vascular endothelial growth factor; MMP-9, matrix metalloproteinase 9; IFN-α, interferon alfa; sIL-2R, soluble interleukin-2 receptor.

*HR indicates relative increase in risk of progression for a patient with a two-fold increase in CAF concentration from baseline compared with a patient with no increase in CAF concentration.

†Treatment $\times$ change-in-CAF interaction was significant ($P < .05$).

‡All associations with PFS were significant ($P < .05$), except day 43 IL-8 ($P = .059$) and IFN-α ($P = .068$), which had significant interactions.

indicate responsiveness or resistance to therapy earlier than imaging studies.

The finding that chemotherapy and vandetanib treatment are associated with distinct changes in the CAF profile has a number of potentially important implications for biomarker development as well as understanding the biologic effects of these agents. First, it suggests that for each drug (or class of drugs), it may be possible to identify specific CAFs whose changes during treatment may serve as pharmacodynamic and/or efficacy markers. In the case of vandetanib monotherapy, we noted a significant decrease in sVEGFR-2 and increase in VEGF by D43, consistent with a previous report.[28] Similar sVEGFR-2 and VEGF changes have been reported in patients with a variety of solid tumor types treated with other VEGFR TKIs and seem to be a pharmacologic class effect.[15,16,22,29-32] Of note, in the VCP arm, reciprocal changes in sVEGFR-2 and VEGF were not observed, suggesting that the effect of chemotherapy on CAF changes dominated over that of vandetanib. The underlying molecular mechanisms of these VEGF and sVEGFR-2 changes are not fully understood.[17] Ebos et al[16] recently showed that the changes in VEGF and sVEGFR-2 in human tumor xenograft-bearing and non–tumor-bearing mice treated with sunitinib occurred through a systemic, multiorgan, tumor-independent mechanism that correlated with the optimal antitumor dose of sunitinib. Therefore, these VEGF and sVEGFR-2 changes have the potential to serve as pharmacodynamic biomarkers to guide optimal biologic dosing.

A number of studies have analyzed for associations between VEGF and/or sVEGFR-2 changes during treatment and clinical outcome, with conflicting findings.[18-22,33,34] Although two phase II studies of VEGFR TKIs for renal cell carcinoma (sunitinib[21] and pazopanib[33]) reported associations between tumor response and plasma VEGF/VEGFR-2 changes, there were no correlations detected between patient outcome and VEGF/sVEGFR-2 changes in a large, randomized, phase III study of sorafenib versus placebo for renal cell carcinoma.[34] In the current study, there was a trend toward inferior PFS with an increase in VEGF at D8 in the vandetanib monotherapy arm, but there was no association between PFS and a change in VEGF levels in the CP and VCP arms. It is noteworthy that neither changes in VEGF nor sVEGFR-2 correlated with outcome in the chemotherapy-containing arms of this study, suggesting that their potential utility as predictors of clinical benefit may be specific for VEGF pathway inhibitors alone.

The pattern of CAF changes over time in the CP and VCP arms was distinct from that in the vandetanib arm, with several CAFs undergoing maximal changes at D8 and returning toward baseline levels by D22. These included significant decreases in MMP-9, IL-12, and IL-1RA and increase in MCP-1 at D8. The effects of chemotherapy on the circulating concentrations of these four CAFs have not been previously reported. Leukocytes are major sources of IL-12, IL-1RA, and MMP-9, and the decrease of these CAFs at D8 may mirror changes in leukocyte levels with cytotoxic chemotherapy.

The biologic consequences of the distinct treatment-induced CAF changes remain to be determined, but preclinical studies suggest that they may have significant effects on both the host and tumor. For example, paclitaxel-induced increases in the chemokine stromal cell-derived factor-1α were recently found to contribute to the mobilization of circulating endothelial progenitors, increased angiogenesis, and tumor growth in a murine lung cancer model.[23] In this study, we report, for the first time to our knowledge, that chemotherapy also induces increases in MCP-1, a known proangiogenic chemokine that regulates VEGF levels and is a key chemoattractant for monocytes.[35] The potential role of MCP-1 in chemotherapy-induced mobilization of proangiogenic mononuclear cells merits further investigation.

We also observed that specific CAF changes were associated with PFS during treatment, and moreover, there were a number of significant treatment $\times$ change-in-CAF interactions, indicating that the correlation differed depending on the treatment. This highlights the need for treatment-specific markers of benefit and suggests potential mechanisms of therapeutic resistance that merit further investigation. For example, greater increases in IL-8 were associated with inferior PFS in the VCP arm. IL-8–mediated angiogenesis was previously identified as a key compensatory angiogenic pathway in a murine model of colorectal cancer.[36] However, an increase in ICAM-1 in the vandetanib arm was predictive of superior PFS, which could perhaps reflect shedding of ICAM-1 secondary to treatment-induced tumor endothelial cell death. It is particularly interesting that an increase in MMP-9 in the CP arm was associated with inferior PFS. A similar

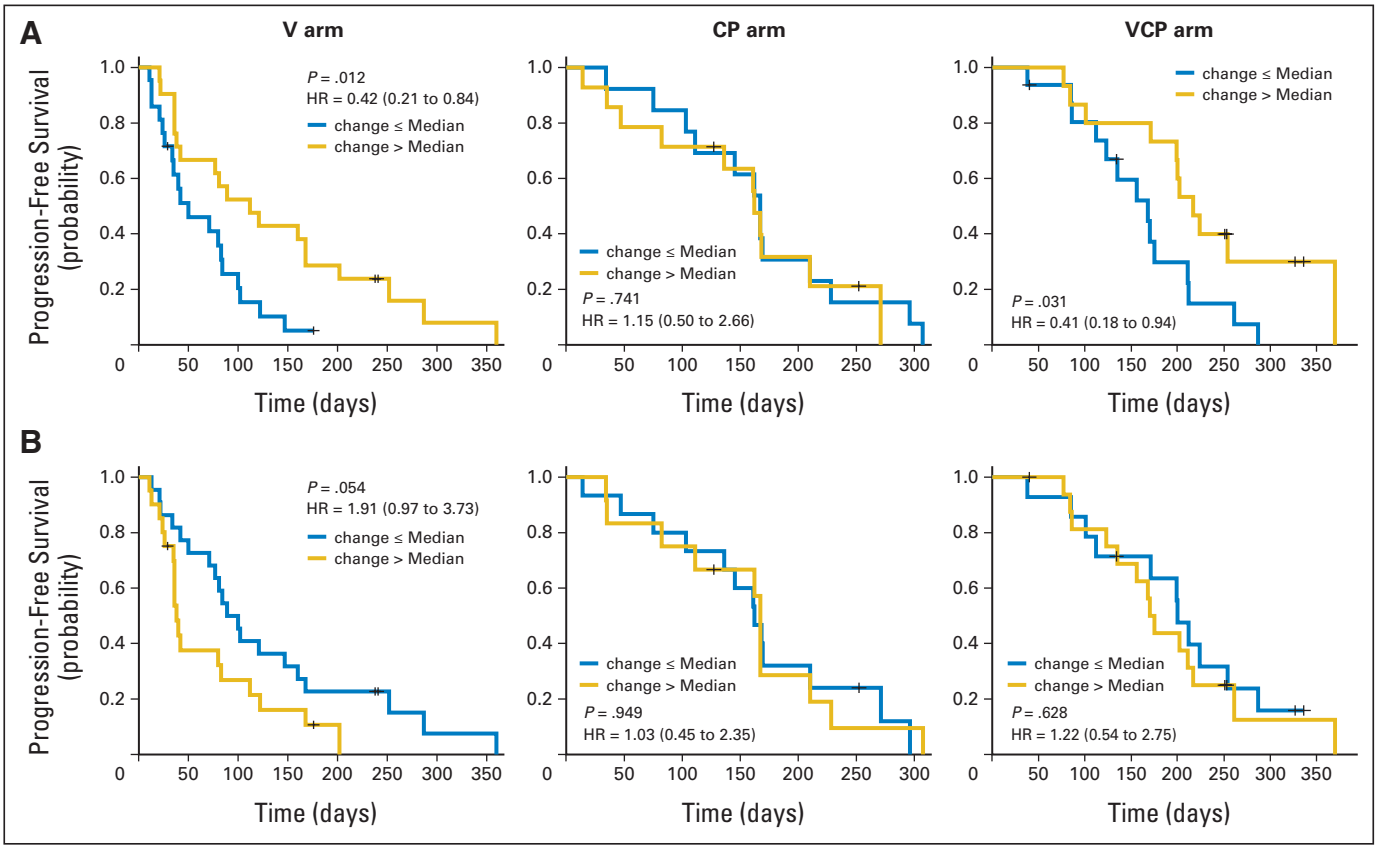

Fig 2. Kaplan-Meier curves of progression-free survival (PFS) based on extent of change in (A) intercellular adhesion molecule 1 (ICAM-1) and (B) vascular endothelial growth factor (VEGF) concentrations (≤ median indicates increase ≤ the median increase in concentration of that cytokine and angiogenic factor [CAF]; > median indicates increase > the median increase in concentration of that CAF). Note that the *P* values are from a log-rank test for the comparison of the Kaplan-Meier curves, whereas the *P* values shown in the text are from a Cox model with the change of the markers as continuous variables adjusting for sex and smoking status. Change in ICAM-1 at day 8 by treatment interaction, $P = .021$; change in VEGF at day 8 by treatment interaction, $P = .009$. V, vandetanib; CP, carboplatin and paclitaxel; VCP, vandetanib, carboplatin, and paclitaxel; HR, hazard ratio.

association between adverse outcome and increasing serum MMP-9 concentration was reported in a study of 116 consecutive patients treated with gemcitabine and cisplatin for NSCLC.[37] MMP-9 has multiple proangiogenic functions, including the degradation of collagen in basement membrane that facilitates endothelial cell migration and liberation of other proangiogenic growth factors, including VEGF.[38,39] MMP-9 delivered to the tumor site by proangiogenic bone marrow–derived cells (BMDCs) has been shown to be critical for tumor neovascularization and BMDC recruitment.[40-43] In light of these data, the association between an increased risk of tumor progression and an increase in MMP-9 could reflect a greater recruitment of BMDCs to tumor sites in these patients, resulting in tumor neovascularization and growth.

Although other researchers have considered the effects of anticancer treatments on CAFs, they have generally evaluated a more limited number of markers in retrospectively identified cohorts of patients or single-arm clinical trials. To our knowledge, this analysis of CAF changes over time considers the largest number of CAFs to date and is one of only a few to assess correlations between modulation of blood-based biomarkers and patient outcome in a prospective, randomized clinical trial using both standard chemotherapy and a targeted agent. In 113 of the 878 participating patients in the randomized phase II/III Eastern Cooperative Oncology Group 4599 study of CP with or without bevacizumab for the first-line treatment of stage IIIB or IV NSCLC, Dowlati et al[44] analyzed the changes during treatment of the following three CAFs: bFGF, ICAM-1, and E-selectin. They found an association between relative stability of E-selectin at week 7 and greater survival benefit from CP plus bevacizumab compared with CP. We found no relationship between E-selectin change/stability and PFS in our study. This discrepancy may reflect differences in the mechanisms of action of bevacizumab and vandetanib, or it could simply be a result of modest patient numbers in both studies.

It is important to note that the CAF analyses reported here are exploratory, and the number of patients is modest. Therefore, it is possible that some of the observed marker changes and their associations with outcome occurred by chance or that we failed to detect clinically relevant changes in markers as a result of a lack of statistical power. Nevertheless, it is notable that we found the same VEGF and sVEGFR-2 changes in the vandetanib arm that have been previously reported with vandetanib and other similar agents.[16,28]

We have shown that vandetanib and chemotherapy are associated with distinct patterns of CAF changes during treatment. Such patterns of CAF changes may provide insight into the biologic effects of these agents and suggest potential mechanisms of resistance and novel therapeutic combinations that merit further investigation.

Furthermore, we have identified changes in CAFs that were associated with PFS benefit. These markers were drug specific because changes in no single marker were associated with outcome in all three treatment arms. On the basis of these findings, additional analyses are planned in ongoing phase III studies of vandetanib in NSCLC to potentially validate these findings and identify other novel markers of activity and clinical benefit.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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