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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Biomarkers Predicting Outcome in Patients with Advanced Renal Cell Carcinoma: Results from Sorafenib Phase III Treatment Approaches in Renal Cancer Global Evaluation Trial

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Abstract

Purpose: Plasma proteins [vascular endothelial growth factor (VEGF), soluble VEGF receptor 2 (sVEGFR-2), carbonic anhydrase IX (CAIX), tissue inhibitor of metalloproteinase 1 (TIMP-1), and Ras p21] and one tumor gene (*VHL*) were analyzed to identify prognostic biomarkers or indicators of response to sorafenib in a subset of patients enrolled in the Treatment Approaches in Renal Cancer Global Evaluation Trial.

Experimental Design: Nine hundred three patients with advanced renal cell carcinoma (RCC) were randomized to 400 mg sorafenib twice a day or placebo. Samples collected at baseline and after 3 and 12 weeks were subjected to enzyme-linked immunosorbent assays. *VHL* exons were sequenced from tumor biopsies.

Results: Baseline biomarker data were available for VEGF ($n = 712$), sVEGFR-2 ($n = 713$), CAIX ($n = 128$), TIMP-1 ($n = 123$), Ras p21 ($n = 125$), and *VHL* mutational status ($n = 134$). Higher Eastern Cooperative Oncology Group performance status (ECOG PS) score correlated with elevated baseline VEGF ($P < 0.0001$) and a higher incidence of *VHL* mutations ($P = 0.008$), whereas higher Memorial Sloan-Kettering Cancer Center (MSKCC) score correlated with elevated VEGF ($P < 0.0001$), CAIX ($P = 0.027$), and TIMP-1 ($P = 0.0001$).

Univariable analyses of baseline levels in the placebo cohort identified VEGF ($P = 0.0024$), CAIX ($P = 0.034$), TIMP-1 ($P = 0.001$), and Ras p21 ($P = 0.016$) as prognostic biomarkers for survival. TIMP-1 remained prognostic for survival in a multivariable analysis model ($P = 0.002$) that also included ECOG PS, MSKCC score, and the other biomarkers assayed. In the placebo cohort, TIMP-1 ($P < 0.001$) and Ras p21 ($P = 0.048$) levels increased at 12 weeks. In the sorafenib cohort, VEGF levels increased at 3 and 12 weeks of treatment (both weeks $P < 0.0001$), whereas sVEGFR-2 (both weeks $P < 0.0001$) and TIMP-1 levels ($P = 0.002$, week 3; $P = 0.006$, week 12) decreased.

Conclusions: VEGF, CAIX, TIMP-1, and Ras p21 levels were prognostic for survival in RCC patients. Of these, TIMP-1 has emerged as being independently prognostic. *Clin Cancer Res; 16(19); 4853–63. ©2010 AACR.*

The Von Hippel-Lindau (*VHL*) gene, commonly mutated in renal cell carcinoma (RCC), is associated with clear cell carcinoma, the most common RCC histology (1–4). Under normoxic conditions, wild-type VHL protein binds to hypoxia-inducible factor, a transcription factor that promotes cell growth and survival under hypoxic conditions, and targets hypoxia-inducible factor for proteosomal degradation (4–6). The loss of VHL protein leads to hypoxia-inducible factor transcriptional activity, including production of carbonic anhydrase IX (CAIX), a tissue hypoxia marker, and growth and angiogenesis factors such as vascular endothelial growth factor (VEGF), transforming growth factor α (TGF-α), erythropoietin, and platelet-derived growth factor (PDGF; ref. 2). The binding of these growth factors to their cognate tyrosine kinase receptors drives cell proliferation and angiogenesis.

Targeted therapies that prevent tyrosine kinase signaling induced by these growth factors have proven efficacious in the treatment of RCC (7–9). Sorafenib is an orally active multikinase inhibitor that targets the intracellular serine/threonine kinase Raf-1 (10) and the receptor tyrosine kinases VEGFR, PDGFR receptor β (PDGFR-β), c-KIT, RET, and FLT-3 (11, 12). Sorafenib is able to directly target both tumor cell proliferation and angiogenesis through the role of these factors in aspects of tumor growth and blood vessel formation (10). Components of these proliferative and proangiogenic signaling pathways, some of which are detectable in plasma, may provide sensitive indicators of tumor activity and response to treatment in RCC.

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Translational Relevance

Identification of biomarkers correlating with outcome in patients with renal cell carcinoma (RCC) may help determine patient prognosis, identify patients most likely to benefit from specific treatments, help monitor response to treatment, and guide clinicians in designing personalized treatment strategies for their patients. Our results indicate that baseline levels of plasma VEGF, CAIX, TIMP-1, and Ras p21 have a prognostic relationship with survival in this subset analysis of patients with RCC, and of these, TIMP-1 has emerged as independently prognostic. These data suggest that such a marker may have value as a prognostic element in RCC staging systems.

Multiple preclinical studies have shown the importance of VEGF in driving angiogenic processes through its cognate receptors VEGFR-1, VEGFR-2, and VEGFR-3 (9). VEGFR-2 signaling in particular has been shown to be a major driver of tumor angiogenesis (9, 13). Ras is an intracellular signaling molecule that plays an essential role in transmitting signals downstream of receptor tyrosine kinases (14). Mutational activation of Ras proteins promotes tumorigenesis by disturbing cellular processes, including cell cycle progression, cell proliferation, survival, and migration (15). In RCC, unregulated activation of Raf kinases and receptor tyrosine kinases through mechanisms such as point mutations or overexpression can induce Ras p21 activation and subsequent upregulation of the downstream Raf/MEK/ERK pathway (2, 16), making Ras p21 potentially useful as a prognostic marker in patients.

Other signaling pathways involved in metastasis and tumor microenvironment interactions, including CAIX and tissue inhibitors of metalloproteinases (TIMP), may also have value as prognostic indicators. CAIX regulates intracellular pH in response to hypoxia (17, 18) and has been investigated as a prognostic factor for survival in patients with metastatic RCC (19). CAIX is highly expressed in advanced RCC but is not expressed in normal kidney, suggesting that it may be a useful marker of tumor progression (19). An imbalance of TIMPs and matrix metalloproteinase enzymes leading to an excess of proteolytic degradation of the extracellular matrix and basement membrane has also been linked to tumor invasion (20). Recent evidence suggests that TIMPs play a complex multifunctional role that includes growth-promoting abilities, depending on the tissue microenvironment (21).

In the phase III Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET), sorafenib doubled progression-free survival (PFS), showed a significant benefit in overall survival (OS) compared with placebo, and had a manageable safety profile (7, 22) in patients with advanced RCC. An additional objective of TARGET was to explore candidate biomarkers for prognosis and sorafenib benefit in patients with advanced RCC. Plasma levels of VEGF, soluble (s)VEGFR-2, CAIX, TIMP-1, and Ras p21 were assayed at baseline and after 3 and 12 weeks of treatment. Tumor *VHL* mutational status was determined in archival tumor samples. We report the results of these exploratory analyses here.

Table 1. Analysis of baseline biomarker levels and clinical/demographic variables

Demographic variable	Subset of patients with baseline data available for CAIX, TIMP-1, and/or Ras *n* (%)	Baseline CAIX (*n* = 128)			Baseline TIMP-1 (*n* = 123)		
		Mean	Median	*P*	Mean	Median	*P*
Sex							
Male	95 (74)	579.1	282.2	0.625	364.0	330.0	0.040
Female	33 (26)	675.6	312.9		441.3	351.2	
Age (y)							
<65	88 (69)	594.0	279.3	0.863	383.7	333.1	0.970
≥65	40 (31)	626.1	334.1		385.0	332.7	
MSKCC score*							
Low	70 (55)	432.2	247.8	0.027	325.3	285.7	0.0001
Intermediate	58 (45)	811.3	390.5		454.5	407.4	
ECOG PS							
0	58 (45)	438.7	236.5	0.075	370.5	309.5	0.549
1	69 (54)	748.1	346.9		390.3	335.5	
2	1 (1)	ND	ND		ND	ND	

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Materials and Methods

Patients and samples

The TARGET design has been previously described (7). Briefly, eligible patients with advanced RCC were randomized (n = 903) to receive either 400 mg sorafenib twice a day (n = 451) or placebo (n = 452). The majority of patients enrolled (>99%) had RCC with clear-cell histology. The primary end point was OS. Secondary end points included PFS, response rate, and patient-reported outcomes.

Plasma samples were collected at baseline and after 3 and 12 weeks of treatment. Blood samples (10 mL) were drawn at each time point using venipuncture or through a porta-catheter into a Vacutainer (BD) containing potassium EDTA and gently inverted. Blood samples were centrifuged in a refrigerated (4°C) centrifuge for 10 minutes to separate the plasma within 10 to 15 minutes after collection. Plasma samples were frozen upright at ≤−70°C within 20 minutes of centrifugation and kept frozen until ready for shipment to the sponsor. Archival formalin-fixed, paraffin-embedded tumor biopsy samples were collected for *VHL* mutational analysis.

Biomarker assays

Commercially available enzyme-linked immunosorbent assay (ELISA) kits for VEGF (detecting VEGF-A; R&D Systems; ref. 23), sVEGFR-2 (R&D Systems; ref. 24), CAIX (detecting the extracellular domain; Siemens Medical Solutions Diagnostics; ref. 25), TIMP-1 (Siemens Medical Solutions Diagnostics; ref. 26), and Ras p21 (detecting H-Ras, K-Ras, and N-Ras; Siemens Medical Solutions Diagnostics; ref. 27) were used per the manufacturers' specifications to measure protein levels in plasma. Laboratory personnel performing the assays and reporting the data were blinded to the trial outcome.

Sequencing of the *VHL* gene

DNA was isolated from formalin-fixed, paraffin-embedded tumor samples using Qiagen's DNeasy Blood & Tissue Kit (28). The three coding exons of the *VHL* gene were amplified through PCR using the following primers: exon 1, forward—GCGGCGTCCGGCCCGGGTGGTCTGGAT, reverse—TGGGTCGGGCCTAAGCGCCGGGCCCGT; exon 2, forward—GTTTCACCACGTTAGCCAGGA, reverse—GATTGGATACCGTGCCTGACA; exon 3, forward—GTTGGCAAAGCCTCTTGTTCG, reverse—GCCCCTAAACATCACAATGCC. PCR conditions were as follows: 94°C for 10 minutes, followed by 35 to 40 cycles of 94°C for 30 seconds, 70°C for 1 minute, and 72°C for 5 minutes, and lastly 4°C indefinitely. PCR products were purified using Qiagen QIAquick PCR Purification Kit, and sequencing was done using PCR primers on the Applied Biosystems 3700 DNA Analyzer. The resulting sequences were aligned with the wild-type (WT) *VHL* gene sequence (Genbank accession no. NM_000551) to identify mutations.

Outcomes assessed

The outcomes and clinical data used for the present correlative analyses were taken directly from the TARGET database. OS was defined as the time elapsed from

Table 1. Analysis of baseline biomarker levels and clinical/demographic variables (Cont'd)

Baseline Ras p21 (n = 125)			Baseline sVEGFR-2 (n = 713)				Baseline VEGF (ref. 22; n = 712)			
Mean	Median	P	n (%)	Mean	Median	P	n (%)	Mean	Median	P
3,302.9	2,456.2	0.540	514 (72)	11,763.61	11,644.54	0.082	509 (72)	206	126	0.760
3,668.7	3,166.2		199 (28)	11,373.69	10,972.33		199 (28)	212	137	
3,528.4	2,898.9	0.452	498 (70)	11,868.00	11,669.30	0.001	496 (70)	214	137	0.290
3,105.6	2,412.0		215 (30)	11,160.91	10,923.37		212 (30)	193	122	
3,096.1	2,500.4	0.207	364 (51)	11,588.07	11,364.66	0.523	362 (51)	159	102	<0.0001
3,754.9	3,332.8		349 (49)	11,724.36	11,594.53		346 (49)	258	161	
2,919.8	2,544.6	0.113	350 (49)	11,791.26	11,566.99	0.429	347 (49)	176	108	<0.0001
3,744.5	3,011.7		356 (50)	11,524.02	11,364.66		354 (50)	233	143	
ND	ND		7 (1)	11,481.22	10,787.09		7 (1)	526	161	

Abbreviation: ND, not done.

*MSKCC score is based on three risk factors: ECOG PS ≥2 (or Karnofsky performance status <80%), low hemoglobin, and high corrected calcium. Low MSKCC score is defined as having none of these risk factors; medium as having one risk factor; and high as having two to three risk factors (29).

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Table 2. Tumor *VHL* mutational status analysis done using archival biopsies

Clinical/demographic variable	Patients with all three VHL exons sequenced (*n* = 48)		Patients included in correlative analysis (*n* = 68)		
	WT,* *n* (%)	Mut,† *n* (%)	WT,* *n* (%)	Mut,† *n* (%)	*P*
Total no. of patients	33 (100)	15 (100)	33 (100)	35 (100)	
Sex					
Male	26 (79)	11 (73)	26 (79)	24 (69)	0.340
Female	7 (21)	4 (27)	7 (21)	11 (31)	
Age (y)					
<65	23 (70)	13 (87)	23 (70)	25 (71)	0.876
≥65	10 (30)	2 (13)	10 (30)	10 (29)	
MSKCC score‡					
Low	17 (52)	8 (53)	17 (52)	20 (57)	0.641
Intermediate	16 (48)	7 (47)	16 (48)	15 (43)	
ECOG PS					
0	22 (67)	5 (33)	22 (67)	12 (34)	0.008
1	11 (33)	10 (67)	11 (33)	23 (66)	
2	0 (0)	0 (0)	0 (0)	0 (0)	

Abbreviations: WT, wild-type; Mut, mutated.

*Includes patients of both the sorafenib and placebo cohorts for whom all three *VHL* exons were sequenced successfully. Note that the frequency of *VHL* coding region mutations must be calculated using a population in which all three exons have been sequenced successfully.

†Includes patients of both the sorafenib and placebo cohorts for whom one or more *VHL* exons were sequenced and one or more exons were determined to harbor a mutation. Note that it was not necessary to sequence all three exons to categorize the coding region as harboring a mutation; if only one exon was sequenced and harbored a mutation, then the gene was classified as mutated. This classification method was used for the *VHL* correlative analysis to increase the *n* available for statistical analysis.

‡MSKCC score is based on three risk factors: ECOG PS ≥2 (or Karnofsky performance status <80%), low hemoglobin, and high corrected calcium. Low MSKCC score is defined as having none of these risk factors; medium as having one; and high as having two to three (29).

randomization to death from any cause, and PFS was defined as the time from randomization to disease progression based on radiologic or clinical assessment or death (whichever occurred earlier). A preplanned interim PFS analysis showed that treatment with sorafenib doubled PFS compared with placebo, allowing for crossover of placebo patients to sorafenib (7, 22). The investigator-assessed PFS data used for biomarker analyses were collected precrossover (data cutoff May 2005), and the OS data used for biomarker analyses were 5 months post-crossover (data cutoff November 2005). The 5-month postcrossover OS data set was chosen for the biomarker correlative analyses to minimize the number of censored OS data points in the data set, increasing the power of the statistical analyses.

Statistical analyses

Patients with biomarker data available for at least one time point were included in statistical analyses. Patients missing a biomarker value for a given time point were excluded from analyses of that biomarker at that time point. Evaluation of the prognostic value of the biomarker was done initially using univariable Cox and Kaplan-Meier analyses, followed by multivariable Cox proportional hazard models. The multivariable models included baseline biomarker value(s) and two clinical variables shown to be prognostic in the overall study population [Eastern Cooperative Oncology Group performance status (ECOG PS) and Memorial Sloan-Kettering Cancer Center (MSKCC) score]. MSKCC score for previously treated patients with RCC is based on three risk factors: ECOG PS ≥2 (or Karnofsky performance status <80%), low hemoglobin, and high corrected calcium. Low MSKCC score is defined as having none of these risk factors; medium as having one; and high as having two to three (29). The effect of sorafenib treatment on biomarker levels was determined with one-way ANOVA tests. The relationship between baseline biomarker levels and sorafenib benefit and between change in biomarker levels and patient outcome was examined using proportional hazards models with an interaction term. Because of the exploratory nature of the biomarker analyses, *P* values were not corrected for multiplicity of testing. *P* values reported should be interpreted as a strength of evidence measure and not as a declaration of statistical significance.

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Results

Patient characteristics and baseline biomarkers

Valid baseline plasma samples were received, and the resulting evaluable ELISA data were available from a total of 712 patients for VEGF and 713 for sVEGFR-2. CAIX, TIMP-1, and Ras p21 were evaluated as an exploratory analysis of a subset of these patients, with ELISA data generated for 128 patients for CAIX, 123 for TIMP-1, and 125 for Ras p21. Valid tumor samples for *VHL* mutational status analysis were received, and at least one of the three *VHL* coding exons was sequenced successfully for 134 patients.Baseline characteristics in the biomarker subpopulations were similar to those in the overall TARGET population (Table 1). Baseline characteristics of the VEGF subpopulation were described previously by Escudier et al. (22) and are also demographically similar to the overall study population. The majority of patients were male (~72–74%), under 65 years of age (~70%), and had an ECOG PS of ≤1 (99%); 51% to 55% of patients also had a low MSKCC score (Table 1; ref. 22). In addition, 31.3% of available patients (n = 48; includes only patients from whom all three exons were sequenced successfully) were found to harbor mutations in the *VHL* gene.

Analysis of baseline plasma biomarker levels as a function of demographic variables (Table 1; ref. 22) indicated that patients with intermediate MSKCC scores had significantly higher baseline VEGF (P < 0.0001),

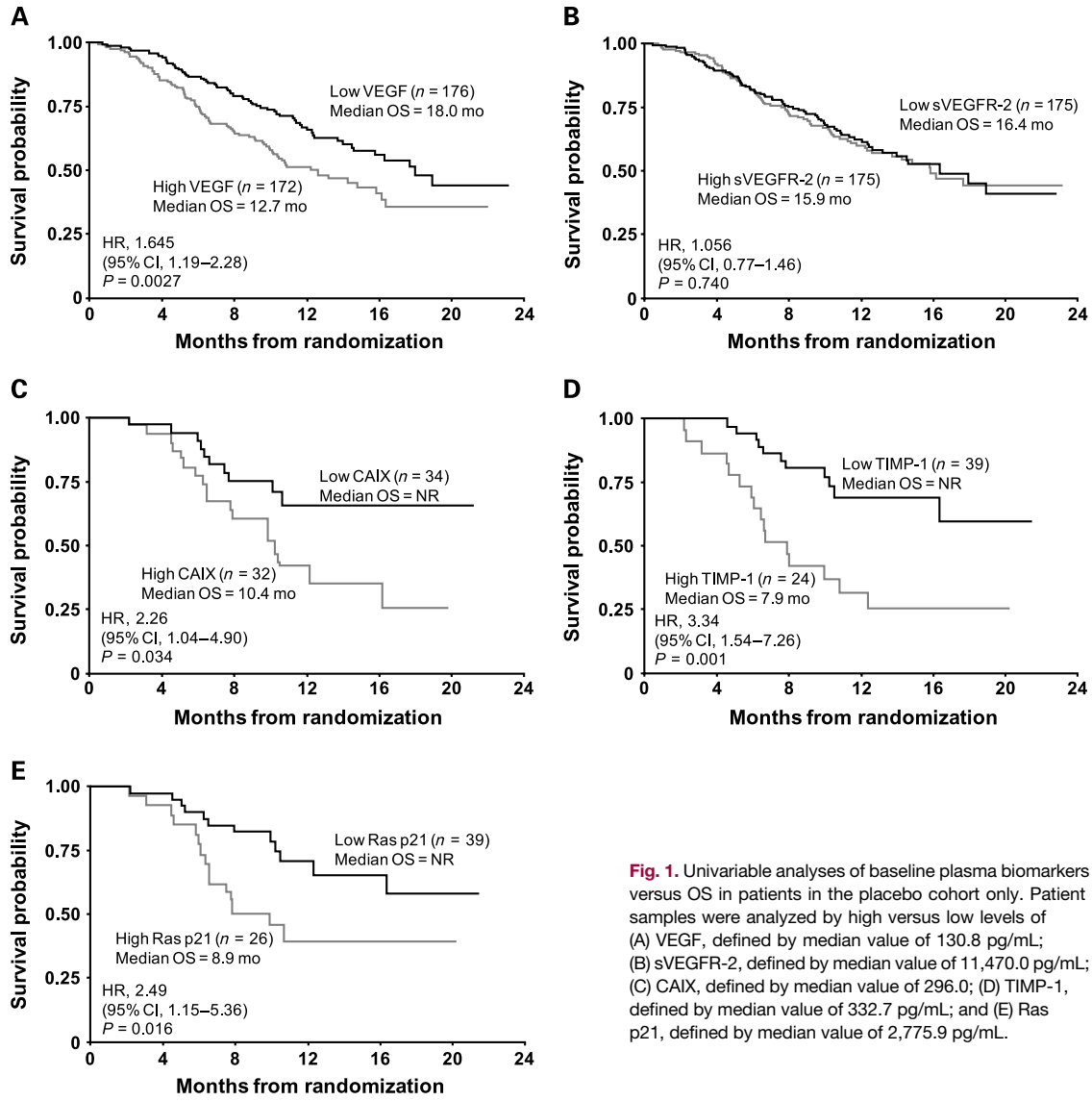

Fig. 1. Univariable analyses of baseline plasma biomarkers versus OS in patients in the placebo cohort only. Patient samples were analyzed by high versus low levels of (A) VEGF, defined by median value of 130.8 pg/mL; (B) sVEGFR-2, defined by median value of 11,470.0 pg/mL; (C) CAIX, defined by median value of 296.0; (D) TIMP-1, defined by median value of 332.7 pg/mL; and (E) Ras p21, defined by median value of 2,775.9 pg/mL.

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Table 3. Multivariable analysis to identify baseline plasma biomarkers independently prognostic for outcome

	OS			PFS		
	P	HR* (95% CI)	Parameter estimate	*P*	HR* (95% CI)	Parameter estimate
MSKCC score†	0.9984	0.999 (0.38–2.60)	–0.0009895	0.4653	0.764 (0.37–1.57)	–0.26899
ECOG PS‡	0.1956	1.863 (0.73–4.78)	0.62223	0.0635	1.923 (0.96–3.84)	0.65390
VEGF	0.2644	0.998 (1.00–1.00)	–0.00152	0.6772	1.00 (1.00–1.00)	0.0003850
VEGFR-2	0.4044	1.000 (1.00–1.00)	0.0000927	0.6315	1.00 (1.00–1.00)	0.0000379
CAIX	0.6643	1.00 (1.00–1.00)	0.0001874	0.8252	1.00 (1.00–1.00)	0.0000773
Ras p21	0.6832	1.00 (1.00–1.00)	0.0000410	0.5065	1.00 (1.00–1.00)	–0.0000495
TIMP-1	0.0020	1.00 (1.00–1.00)	0.00388	0.1481	1.00 (1.00–1.00)	0.00141

NOTE: Included placebo patients with data available for VEGF, VEGFR-2, CAIX, Ras p21, and TIMP-1 (n = 59). Biomarker values were analyzed as continuous variables.

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

*Hazard ratio when a continuous variable increases by 1 unit.

†MSKCC score is based on three risk factors: ECOG PS ≥2 (or Karnofsky performance status <80%), low hemoglobin, and high corrected calcium. Low MSKCC score is defined as having none of these risk factors; medium as having one; and high as having two to three (29).

‡ECOG PS 1 and 2 are combined in the analysis.

CAIX (P = 0.027), and TIMP-1 (P = 0.0001) than those with low MSKCC scores. Baseline levels of sVEGFR-2, Ras p21, and *VHL* mutational status did not differ significantly between patients with low and intermediate MSKCC scores. Patients with poor ECOG PS scores had significantly higher baseline VEGF levels (P < 0.0001; ref. 22) and a higher incidence of *VHL* gene mutations (P = 0.008; Table 2). Baseline levels of sVEGFR-2, CAIX, TIMP-1, and Ras p21 did not significantly correlate with ECOG PS score.

Prognostic value of baseline biomarkers

Univariable analysis of the placebo cohort identified baseline plasma levels of VEGF (n = 348; P = 0.0024), CAIX (n = 66; P = 0.034), TIMP-1 (n = 63; P = 0.001), and Ras p21 (n = 65; P = 0.016) as prognostic factors for OS in RCC (Fig. 1). Patients with high (>median) baseline VEGF (>130.8 pg/mL), CAIX (>296.0 pg/mL), TIMP-1 (>332.7 pg/mL), or Ras p21 (>2775.9 pg/mL) had shorter survival times than patients with low (<median) baseline levels of these factors. Similar univariable analyses of PFS revealed that elevated levels of Ras p21 (hazard ratio, 1.84; median PFS, low and high Ras patients, 4.7 and 2.8 months, respectively; P = 0.040) and VEGF (P < 0.01; ref. 22) also correlated with shorter PFS in placebo-treated patients. sVEGFR-2 (n = 350) did not show a prognostic relationship with either OS (Fig. 1) or PFS (data not shown).

The prognostic value of VEGF, VEGFR-2, CAIX, TIMP-1, and Ras p21 was further explored using multivariable analyses. Multivariable analysis of all available patients with baseline VEGF data has been published previously (22) and revealed that VEGF retained independent prognostic value in placebo patients for both OS (P = 0.042) and PFS (P = 0.023). A further multivariable analysis was done in the subset of patients (n = 59; placebo patients only) who had data available for all five plasma biomarkers assayed (Table 3). Inclusion of these biomarkers plus ECOG PS and MSKCC score in the same model revealed that only TIMP-1 remained independently prognostic for survival (P = 0.002).

Value of baseline biomarkers in predicting treatment benefit

To investigate the potential relationship between baseline biomarkers and sorafenib benefit, Cox analyses including patients from both the sorafenib- and placebo-treated cohorts were done to examine the biomarker treatment interaction. The effect of baseline VEGF level on sorafenib benefit has been described previously (22); results suggest that patients with elevated VEGF (>75th percentile) may experience greater benefit from sorafenib treatment (in terms of PFS) than those with low VEGF. Similar analyses of sVEGFR-2, CAIX, TIMP-1, Ras p21, and *VHL* mutational status revealed no relationship between these biomarkers and sorafenib benefit (Table 4).

Changes in biomarker levels during treatment

The changes in biomarker levels from baseline to weeks 3 and 12 of treatment were analyzed to identify biomarkers that are potentially modulated by sorafenib treatment or by the course of disease (Table 5). Baseline VEGF (n = 712) and sVEGFR-2 (n = 713) levels did not differ significantly between the sorafenib and placebo cohorts. Mean VEGF levels increased significantly in sorafenib-treated patients at weeks 3 and 12 (P < 0.0001 for both), but did not change significantly in those treated with placebo. The VEGF response during treatment also differed

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Table 4. Analysis of interaction between baseline biomarkers and sorafenib treatment effect

Biomarker	No. of patients	Analysis description/ cutoff used to define low and high biomarker levels	OS			PFS		
			HR (95% CI), low biomarker or no mutation group	HR (95% CI), high biomarker or mutation group	*P*	HR (95% CI), low biomarker or no mutation group	HR (95% CI), high biomarker or mutation group	*P*
VEGF (22)*	712	Initial analysis: median, all available data	0.89 (0.63–1.27)	0.81 (0.60–1.09)	0.327	0.64 (0.49–0.83)	0.48 (0.38–0.62)	0.096
		Further exploration of data						
	349	•25th percentile, first half of data (hypothesis generation)	ND	ND	ND	0.92 (0.57–1.49)	0.42 (0.31–0.56)	0.001
	363	•25th percentile, second half of data (hypothesis confirmation)	ND	ND	ND	0.75 (0.42–1.32)	0.53 (0.40–0.71)	0.575
	349	•75th percentile, first half of data (hypothesis generation)	ND	ND	ND	0.58 (0.43–0.78)	0.27 (0.15–0.46)	0.020
	363	•75th percentile, second half of data (hypothesis confirmation)	ND	ND	ND	0.69 (0.51–0.94)	0.33 (0.20–0.55)	0.023
sVEGFR-2	713	Median	0.90 (0.65–1.24)	0.82 (0.60–1.14)	0.598	0.56 (0.43–0.72)	0.55 (0.42–0.71)	0.981
CAIX	128	Median	0.98 (0.40–2.42)	0.71 (0.35–1.45)	0.54	0.52 (0.26–1.03)	0.62 (0.33–1.16)	0.72
TIMP-1	123	Median	0.76 (0.26–2.19)	0.58 (0.29–1.15)	0.67	0.58 (0.26–1.30)	0.54 (0.29–0.99)	0.83
Ras	125	Median	1.23 (0.50–3.04)	0.53 (0.25–1.13)	0.18	0.74 (0.37–1.52)	0.41 (0.22–0.79)	0.27
VHL mutational status	116	Exon 1 only†, WT vs mut	0.74 (0.39–1.40)	1.08 (0.32–3.59)	0.634	0.55 (0.34–0.89)	0.39 (0.15–1.04)	0.356
	83	Exon 2 only‡, WT vs mut	0.56 (0.26–1.22)	0.33 (0.03–3.19)	0.536	0.45 (0.26–0.77)	0.89 (0.24–3.34)	0.363
	91	Exon 3 only§, WT vs mut	0.76 (0.40–1.44)	0.00 (0.00–NE)	0.991	0.62 (0.38–1.00)	0.00 (0.00–NE)	0.985
	68	Exon 1, 2, or 3‖, WT vs mut	1.09 (0.36–3.27)	0.88 (0.31–2.48)	0.693	0.52 (0.23–1.19)	0.49 (0.22–1.08)	0.968

Abbreviations: NE, not estimable due to small *n* in one group.

*VEGF levels: 25th percentile = 71 pg/mL; median = 131 pg/mL; 75th percentile = 254 pg/mL.

†Includes all patients in whom *VHL* exon 1 was sequenced successfully.

‡Includes all patients in whom *VHL* exon 2 was sequenced successfully.

§Includes all patients in whom *VHL* exon 3 was sequenced successfully.

‖Gene was designated wild-type only for those patients for whom all three *VHL* exons were sequenced successfully, and all three exons were wild-type (*n* = 33). Gene was designated as mutated in patients for whom one or more *VHL* exons were sequenced and one or more exons were determined to harbor a mutation (*n* = 35).

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Table 5. Baseline biomarker levels and changes in biomarker levels at weeks 3 and 12

Biomarker		Placebo			Sorafenib			Sorafenib vs placebo
		Mean*	Mean Δ from BL, abs (%)†	*P* value for Δ from BL	Mean*	Mean Δ from BL, abs (%)†	*P* value for Δ from BL	*P* value for BL or Δ from BL
			n = 348			*n* = 364		
VEGF (pg/mL), BL (*n* = 712)	Baseline	201.7	NA	NA	212.3	NA	NA	0.548
	Week 3	213.1	8.8 (24.4%)	0.416	279.5	61.5 (93.0%)	<0.0001	0.005
	Week 12	170.4	17.7 (31.8%)	0.084	312.0	117.0 (106.8%)	<0.0001	0.007
			n = 350			*n* = 363		
sVEGFR-2 (pg/mL), BL (*n* = 713)	Baseline	11,555.2	NA	NA	11,754.2	NA	NA	0.332
	Week 3	12,062.9	389.9 (5.1%)	0.010	9,660.4	–2,224.0 (–17.2%)	<0.0001	<0.001
	Week 12	11,778.8	–84.2 (1.8%)	0.695	8,941.9	–3,065.3 (–23.2%)	<0.0001	<0.001
			n = 66			*n* = 62		
CAIX (pg/mL), BL (*n* = 128)	Baseline	430.5	NA	NA	788.7	NA	NA	0.129
	Week 3	391.5	12.7 (3.7%)	0.519	723.7	–113.6 (24.5%)	0.232	0.171
	Week 12	807.8	289.3 (40.4%)	0.077	752.8	–23.42 (17.8%)	0.902	0.187
			n = 63			*n* = 60		
TIMP-1 (pg/mL), BL (*n* = 123)	Baseline	346.1	NA	NA	424.1	NA	NA	0.005
	Week 3	356.5	17.7 (7.4%)	0.429	361.7	–67.4 (–9.5%)	0.002	0.004
	Week 12	406.5	75.9 (21.0%)	<0.001	390.5	–50.1 (–9.4%)	0.006	<0.001
			n = 65			*n* = 60		
Ras p21 (pg/mL), BL (*n* = 125)	Baseline	2,665.9	NA	NA	4,188.0	NA	NA	0.001
	Week 3	2,791.9	187.1 (120.6%)	0.331	3,732.2	–213.4 (42.1%)	0.274	0.146
	Week 12	3,020.6	889.1 (254.7%)	0.048	4,010.4	280.7 (17.1%)	0.264	0.022

Abbreviations: BL, baseline; NA, not applicable; abs, absolute value.

*Mean was calculated for all patients with biomarker data available at this time point.

†Absolute change from baseline was calculated individually for each patient, and then changes for all were averaged.

significantly between the sorafenib and placebo cohorts at both time points assayed (week 3, $P = 0.005$; week 12, $P = 0.007$). Plasma sVEGFR-2 levels decreased in sorafenib-treated patients ($P < 0.0001$ at weeks 3 and 12), and increased marginally at week 3 in patients treated with placebo ($P = 0.010$). The change in sVEGFR-2 differed significantly between the sorafenib- and placebo-treated cohorts at both weeks 3 and 12 ($P < 0.001$ for both time points).

Furthermore, whereas baseline levels of CAIX ($n = 128$) did not differ significantly between the sorafenib and placebo cohorts ($P = 0.129$), baseline TIMP-1 ($n = 123$) and Ras p21 ($n = 125$) differed significantly between treatment arms ($P = 0.005$ and $P = 0.001$, respectively), with higher levels of both factors occurring in the sorafenib cohort (Table 5). TIMP-1 decreased significantly during treatment in the sorafenib cohort at weeks 3 ($P = 0.002$) and 12 ($P = 0.006$), whereas it increased in the placebo cohort at week 12 ($P < 0.001$). These changes in TIMP-1 differed significantly between the sorafenib and placebo cohorts at weeks 3 ($P = 0.004$) and 12 ($P < 0.001$). Although Ras p21 level did not change significantly in the sorafenib cohort, it increased significantly in the placebo cohort at week 12 ($P = 0.048$). The Ras response at week 12 differed significantly between the placebo group and the sorafenib group ($P = 0.022$). Plasma CAIX did not change significantly in either treatment cohort at week 3 or 12.

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No correlations were observed between the magnitude of change in biomarker levels from baseline to week 3 or 12 and outcome (OS or PFS) in sorafenib-treated patients (data not shown).

Discussion

The phase III placebo-controlled TARGET provided an ideal setting in which to examine potential biomarkers of disease prognosis and drug benefit. In the present exploratory analysis, plasma samples from RCC patients were analyzed to examine the potential utility of these biomarkers as prognostic indicators of disease outcome and as predictors of sorafenib benefit.

In a recent publication, we reported that baseline plasma VEGF correlated with MSKCC score and ECOG PS, and was prognostic when analyzed in 348 patients from the placebo group enrolled in TARGET (22). A multivariable analysis that examined VEGF, ECOG PS, and MSKCC score in these placebo patients showed that all three factors were independently prognostic for OS (22). Lastly, exploratory analyses of the relationship between VEGF and sorafenib benefit in 712 TARGET patients suggested that those with levels above the 75th percentile at baseline may benefit more from sorafenib (in terms of PFS) than those with low levels, although sorafenib benefit was apparent in both groups (22).

The current analyses of baseline biomarker levels as a function of demographic variables were conducted to determine if levels of other biomarkers correlated with established prognostic indicators (i.e., MSKCC and ECOG PS scores). Indeed, elevated baseline plasma CAIX, TIMP-1, and the presence of tumor *VHL* mutations were all associated with poor clinical prognostic scores. Baseline biomarkers were further examined in univariable analyses in patients randomized to placebo to determine whether they also offer prognostic value for the natural course of RCC. These analyses indicated that high baseline plasma CAIX, TIMP-1, and Ras p21 were prognostic factors for reduced OS (as well as PFS for Ras p21) in patients with advanced RCC. Analysis of VEGF, CAIX, TIMP-1, and Ras p21 together in 59 patients who had received placebo in TARGET showed that of these prognostic biomarkers, TIMP-1 remained the sole independently prognostic factor for OS in a multivariable model that also included MSKCC score and ECOG PS.

The proangiogenic factor VEGF is known to have increased tumor activity in RCC (1). High serum VEGF-165 (the predominant isoform of VEGF-A in circulation) levels have also been associated with tumor stage, tumor grade, and poor prognosis in RCC (30). Elevated levels of tumor and/or plasma TIMP-1 are associated with poor survival prognosis in a number of tumor types [colorectal (31, 32), gastric (33), breast (34–36), ovarian (37), and lung (38) cancers]. In a number of these studies, plasma TIMP-1 was an independent prognostic factor upon multivariable analysis. Elevated tumor levels of TIMP-1 have also been linked to tumor grade and prognosis in RCC (39), although plasma levels of TIMP-1 have not been similarly reported in RCC. Thus, the current finding that plasma TIMP-1 levels are independently prognostic for survival in previously treated patients with RCC is novel and supports the tumor TIMP-1 findings. Plasma TIMP-1 warrants further study as a prognostic factor for RCC and may provide value when included in prognostic scoring systems for RCC.

Low levels of tumor CAIX have been linked to poor patient prognosis in RCC (19, 40), although other studies have not shown this relationship (41). The present study examined the plasma-detectable shed extracellular domain of CAIX, and, in contrast to the published results on tumor CAIX, elevated CAIX extracellular domain correlated with poor survival. Although the relationship between tumor and plasma levels of CAIX is not understood, the current observation suggests that the level of shed CAIX extracellular domain may reflect relevant tumor biology or disease state.

Elevated serum Ras p21 correlated with poor survival in one study of hematologic malignancies (42). The present finding that high plasma levels of Ras p21 protein correlated with poor survival suggests that circulating Ras reflects relevant tumor biology or disease state.

Plasma levels of VEGF, sVEGFR-2, CAIX, TIMP-1, and Ras p21 were determined at baseline and after 3 and 12 weeks of treatment. Baseline levels of TIMP-1 and Ras p21 were significantly higher in the sorafenib group compared with the placebo group, indicating a potential imbalance between the treatment populations. Nevertheless, plasma levels of VEGF increased significantly in response to sorafenib but remain unchanged in the placebo group, whereas sVEGFR-2 decreased in response to sorafenib. TIMP-1 plasma levels also decreased in the sorafenib cohort but increased in the placebo group (only at 12 weeks). Increases in plasma Ras protein levels were also observed in placebo-treated patients (only at week 12), whereas no significant changes were observed in patients treated with sorafenib. The magnitude of the changes did not correlate with outcome in sorafenib-treated patients for any of the proteins assayed.

The reciprocal changes observed in VEGF and sVEGFR-2 levels following sorafenib treatment are similar to those observed with other antiangiogenic agents (43–47), and collectively are suggestive of a class effect. The lack of correlation observed in the present study between the change in VEGF or sVEGFR-2 and outcome may be due to the systemic nature of the changes, as opposed to a tumor-specific phenomenon. Increases in TIMP-1 and Ras p21 observed in placebo patients (but not in sorafenib patients) during the course of the trial may reflect the advancement of disease.

The results of the present analyses are hypothesis generating, and interpretation should be tempered by the retrospective nature of the biomarker study and the small sample size available for some of the analyses.

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Additionally, the results should be interpreted with caution due to the large number of statistical analyses done. Based on this concern, only one bifurcation cutoff point to separate "high" from "low" biomarker values was analyzed for most plasma biomarkers (the median). The one exception to this is the analysis of VEGF versus sorafenib benefit, for which the median VEGF level was used in the initial analysis, but additional bifurcation cutoffs were also examined. To address the increased risk of generating false-positive signals due to multiple testing, for the analyses of the additional bifurcation points (the 25th and 75th percentiles), the VEGF population was split into two random halves for hypothesis generation and hypothesis confirmation.

Additional study would be required to confirm the clinical relevance and utility of the biomarkers assayed here. In analyses designed to detect predictors of sorafenib benefit in RCC, only VEGF showed any predictive ability (22). Based on the strength of the association between TIMP-1 and prognosis, TIMP-1 should be investigated prospectively as a potential biomarker, such as the MSKCC prognostic factor analysis, in both previously treated and untreated RCC patients.

Disclosure of Potential Conflicts of Interest

C. Pena, M. Shan, C. Lathia, employment, Bayer HealthCare Pharmaceuticals. B. Escudier, advisory board, Bayer, Pfizer, Roche, Novartis, GSK. R. Bukowski, honoraria from speaker's bureau and advisory board, Pfizer, Novartis, Genentech, Bayer.

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References

1. Na X, Wu G, Ryan CK, Schoen SR, di'Santagnese PA, Messing EM. Overproduction of vascular endothelial growth factor related to von Hippel-Lindau tumor suppressor gene mutations and hypoxia-inducible factor-1 α expression in renal cell carcinomas. J Urol 2003;170:588–92.
2. Grandinetti CA, Goldspiel BR. Sorafenib and sunitinib: novel targeted therapies for renal cell cancer. Pharmacotherapy 2007;8:1125–44.
3. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. Lancet 2009;373:1119–32.
4. Kaelin WG, Jr. The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. Clin Cancer Res 2007;13:680–4s.
5. Haase VH. The VHL tumor suppressor: master regulator of HIF. Curr Pharm Des 2009:3895–903.
6. Ivan M, Kondo K, Yang H, et al. HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science 2001;292:464–8.
7. Escudier B, Eisen T, Stadler WM, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 2007;356:125–34.
8. Motzer RJ, Rini BI, Bukowski RM, et al. Sunitinib in patients with metastatic renal cell carcinoma. JAMA 2006;295:2516–24.
9. Rini BI. Vascular endothelial growth factor-targeted therapy in metastatic renal cell carcinoma. Cancer 2009;115:2306–12.
10. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004;64:7099–109.
11. Wilhelm S, Carter C, Lynch M, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nat Rev Drug Discov 2006;5:835–44.
12. Carlomagno F, Anaganti S, Guida T, et al. BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst 2006;98:326–34.
13. Rini BI, Small EJ. Biology and clinical development of vascular endothelial growth factor-targeted therapy in renal cell carcinoma. J Clin Oncol 2005;23:1028–43.
14. Campbell SL, Khosravi-Far R, Rossman KL, Clark GJ, Der CJ. Increasing complexity of Ras signaling. Oncogene 1998;17:1395–413.
15. Giehl K. Oncogenic Ras in tumour progression and metastasis. Biol Chem 2005;386:193–205.
16. Beeram M, Patnaik A, Rowinsky EK. Raf: a strategic target for therapeutic development against cancer. J Clin Oncol 2005;23:6771–90.
17. Pastorekova S, Ratcliffe PJ, Pastorek J. Molecular mechanisms of carbonic anhydrase IX-mediated pH regulation under hypoxia. BJU Int 2008;101:8–15.
18. Chiche J, Ilc K, Laferriere J, et al. Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. Cancer Res 2009;69:358–68.
19. Bui MHT, Seligson D, Han KR, et al. Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. Clin Cancer Res 2003;9:802–11.
20. Bhuvarahamurthy V, Kristiansen G, Johannsen M, Schnorr D, Jung K, Staack A. *In situ* gene expression and localization of metalloproteinases MMP1, MMP2, MMP3, MMP9, and their inhibitors TIMP1 and TIMP2 in human renal cell carcinoma. Oncol Rep 2006;15:1379–84.
21. Stetler-Stevenson WG. Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. Sci Signal 2008;1:re6.
22. Escudier B, Eisen T, Stadler WM, et al. Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J Clin Oncol 2009;27:3312–8.
23. Quantikine [package insert, Cat. No. DVE00]. Minneapolis, MN: R&D Systems, Inc.; 2010.
24. Quantikine [package insert, Cat. No. DVR200]. Minneapolis, MN: R&D Systems, Inc.; 2010.
25. MN/CAIX ELISA [package insert, Item No. 06490035]. Tarrytown, NY: Siemens Healthcare Diagnostics, Inc.; 2008.
26. TIMP-1 ELISA [package insert, Item No. 06489973]. Tarrytown, NY: Siemens Healthcare Diagnostics, Inc.; 2010.
27. Ras p21 ELISA [package insert]. Tarrytown, NY: Siemens Healthcare Diagnostics, Inc.; 2010.
28. Dneasy Blood & Tissue Kit [package insert, Cat. No 69506]. Valencia, CA: Qiagen, Inc.; 2006.

Published OnlineFirst July 22, 2010; DOI: 10.1158/1078-0432.CCR-09-3343

29. Motzer RJ, Bacik J, Schwartz LH, et al. Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. J Clin Oncol 2004;22:454–63.
30. Bukowski R. Prognostic factors for survival in metastatic renal cell carcinoma: update 2008. Cancer 2009;15:2273–81.
31. Yukawa N, Yoshikawa T, Akaike M, et al. Impact of plasma tissue inhibitor of matrix metalloproteinase-1 on long-term survival in patients with colorectal cancer. Oncology 2007;72:205–8.
32. Waas ET, Hendriks T, Lomme RM, Wobbes T. Plasma levels of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 correlate with disease stage and survival in colorectal cancer patients. Dis Colon Rectum 2005;48:700–10.
33. Yoshikawa T, Cho H, Tsuburaya A, Kobayashi O. Impact of plasma tissue inhibitor of metalloproteinase-1 on long-term survival in patients with gastric cancer. Gastric Cancer 2009;12:31–6.
34. Kuvaja P, Talvensaari-Mattila A, Turpeenniemi-Hujanen T. High preoperative plasma TIMP-1 is prognostic for early relapse in primary breast carcinoma. Int J Cancer 2008;123:846–51.
35. Wurtz SO, Moller S, Mouridsen H, Hertel PB, Friis E, Brunner N. Plasma and serum levels of tissue inhibitor of metalloproteinases-1 are associated with prognosis in node-negative breast cancer: a prospective study. Mol Cell Proteomics 2008;7:424–30.
36. Lipton A, Ali SM, Leitzel K, et al. Elevated plasma tissue inhibitor of metalloproteinase-1 level predicts decreased response and survival in metastatic breast cancer. Cancer 2007;109:1933–9.
37. Manenti L, Paganoni P, Floriani I, et al. Expression levels of vascular endothelial growth factor, matrix metalloproteinases 2 and 9 and tissue inhibitor of metalloproteinases 1 and 2 in the plasma of patients with ovarian carcinoma. Eur J Cancer 2003;39:1948–56.
38. Ylisirniö S, Höyhtyä M, Turpeenniemi-Hujanen T. Serum matrix metalloproteinases-2, -9 and tissue inhibitors of metalloproteinases-1, -2 in lung cancer—TIMP-1 as a prognostic marker. Anticancer Res 2000;20:1311–6.
39. Kallakury BV, Karikehalli S, Haholu A, Sheehan CE, Azumi N, Ross JS. Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma. Clin Cancer Res 2001;7:3113–9.
40. Liao SY, Aurelio ON, Jan K, Zavada J, Stanbridge EJ. Identification of the MN/CA9 protein as a reliable diagnostic biomarker of clear cell carcinoma of the kidney. Cancer Res 1997;57:2827–31.
41. Leibovich BC, Sheinin Y, Lohse CM, et al. Carbonic anhydrase IX is not an independent predictor of outcome for patients with clear cell renal cell carcinoma. J Clin Oncol 2007;25:4757–64.
42. Nelli EM, Leitzel K, Ali M, et al. Elevated serum ras level predicts decreased survival in patients with hematologic malignancies [abstract]. 2006 ASCO Annual Meeting Proceedings Part 1. J Clin Oncol 2006;24.
43. Rini BI, Michaelson MD, Rosenberg JE, et al. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. J Clin Oncol 2008;26:3743–8.
44. Burstein HJ, Elias AD, Rugo HS, et al. Phase II study of sunitinib malate, an oral multitargeted tyrosine kinase inhibitor, in patients with metastatic breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol 2008;26:1810–6.
45. Norden-Zfoni A, Desai J, Manola J, et al. Blood-based biomarkers of SU11248 activity and clinical outcome in patients with metastatic imatinib-resistant gastrointestinal stromal tumor. Clin Cancer Res 2007;13:2643–50.
46. Deprimo SE, Bello CL, Smeraglia J, et al. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. J Transl Med 2007;5:32.
47. Ebos JM, Lee CR, Christensen JG, Mutsaers AJ, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. Proc Natl Acad Sci U S A 2007;104:17069–74.

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