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Companion Diagnostic, Pharmacogenomic, and Cancer Biomarkers

Molecular Cancer Therapeutics

Modulation of Circulating Protein Biomarkers in Cancer Patients Receiving Bevacizumab and the Anti-Endoglin Antibody, TRC105

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Abstract

TRC105 is an anti-endoglin antibody currently being tested in combination with VEGF inhibitors. In the phase Ib trial, 38 patients were treated with both TRC105 and bevacizumab (BEV), and improved clinical outcomes were observed, despite the fact that 30 patients (79%) were refractory to prior anti-VEGF therapy. Plasma samples were tested for angiogenic and inflammatory biomarkers at baseline and on-treatment. To provide broader context of this combination biomarker study, direct cross-study comparisons were made to biomarker studies previously conducted in patients treated with either BEV or TRC105 monotherapy. Upon treatment with BEV and TRC105, pharmacodynamic changes in response to both BEV (PlGF increase) and TRC105 (soluble endoglin increase) were noted. In addition, distinct patterns of change were identified (similar, opposing, neutralizing). Similar patterns were observed when the combination elicited similar effects to those observed with monotherapy treatment (i.e., decreases of Ang-2, increases of IL6 and VCAM-1). Opposing patterns were observed when the combination led to opposing effects compared with monotherapy treatment (i.e., TGFβ1, PDGF-AA and PDGF-BB, PAI-1). Lastly, neutralizing patterns were observed when one drug led to increase, whereas the other drug led to decrease, and the combination elicited no overall effect on the marker (i.e., VEGF-A, VEGF-D, and IGFBP-3). Patients achieving partial responses or stable disease from the combination exhibited significantly lower expression of E-Cadherin, HGF, ICAM-1, and TSP-2 at baseline. Taken together, the novel biomarker modulations identified may deepen our understanding of the underlying biology in patients treated with BEV and TRC105 compared with either drug alone. *Mol Cancer Ther; 17(10); 2248–56. ©2018 AACR.*

Introduction

TRC105 is a chimeric IgG1 antibody to endoglin, a type III TGFβ receptor highly expressed on proliferating vascular endothelium in solid tumors (1, 2). Multiple lines of evidence reveal a pivotal role for endoglin in mediating resistance to VEGF inhibitors. Endoglin levels increase on tumor endothelial cells following VEGF inhibition and facilitate tumor growth (3, 4), whereas genetic downregulation or conditional deletion of endoglin reverses resistance to large- and small-molecule inhibitors of the VEGF pathway (5).

TRC105 was well tolerated in a phase I clinical trial, demonstrated a safety profile that was distinct from that of VEGF inhibitors, and exhibited evidence of antitumor activity in advanced cancer patients (6). Biomarker analyses in these patients revealed that TRC105 administration modulated soluble angiogenic factors, including PlGF and VEGF-D, differently than in patients treated with VEGF inhibitors (7, 8). VEGF-A increased following TRC105 treatment (9), suggesting a mechanism of acquired resistance to TGFβ pathway inhibition and a rationale for the dual targeting of endoglin and VEGF. TRC105 has therefore been combined with multiple VEGF inhibitors, including BEV, axitinib, sorafenib, and pazopanib (10). Based on promising phase II data, TRC105 is currently being studied in a pivotal phase III trial with pazopanib in angiosarcoma (NCT02979899).

The first trial testing the combination of TRC105 and BEV was reported in 2014 (11). Of the 38 patients enrolled, 30 patients had received prior BEV treatment. Addition of TRC105 to BEV demonstrated signs of clinical activity, even in patients previously refractory to an anti-VEGF agent: two patients had partial responses (PR) by RECIST evaluation and 18 patients had stable disease (SD).

Identification of soluble biomarkers that predict benefit from the combination would allow enrichment of more responsive patients. To assess many of the key regulators of tumor angiogenesis, inflammation, and matrix remodeling, we developed a multiplex protein array, termed the Angiome (12–15). We have taken a systematic approach to the development of this array, developing high-quality assays that have excellent sensitivity and yield reproducible results. This panel has been continuously optimized to include the most scientifically rational markers based on current knowledge. Importantly, this multiplex assay has recently been reviewed by the NCI Biomarker Review

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Committee and approved as an integrated biomarker in multiple upcoming clinical trials. Applying consistent, fully-validated technologies, such as the Angiome, will hopefully provide more meaningful data across individual trials, leading to better biomarker development.

In this report, we tested the Angiome multiplex array in the 38 patients who received both TRC105 and BEV in this phase Ib trial. We assessed the plasma levels of 36 circulating protein biomarkers at baseline and along the continuum of treatment. Our consistent application of the Angiome across studies allowed for additional comparisons to earlier biomarker analyses in which patients were treated with either TRC105 monotherapy or BEV monotherapy.

Materials and Methods

Patients' enrollment, drug schedule, and sample collection

Between May 2011 and May 2013, 38 patients with advanced or metastatic solid tumors were enrolled and treated with TRC105 (3, 6, 8, and 10 mg/kg/week) and BEV (15 mg/kg every 3 weeks or 10 mg/kg every 2 weeks; ref. 11). Eight patients received both TRC105 and BEV on C1D1, following 3-week cycles, whereas 30 patients received a 7-day BEV lead in monotherapy, followed by a 4-week treatment cycle (Fig. 1). Written-informed consent was obtained from each patient regarding the use of plasma for this correlative analysis. This study was Institutional Review Board–approved and registered with www.clinicaltrials.gov (NCT01332721).

Double-spun, platelet-poor plasma was collected from each patient at baseline, cycle 1 day 8 (C1D8, the end of BEV lead-in monotherapy), C1D15 (coadministration of BEV and TRC105 for 1 week), C2D1 (coadministration of both drugs for 3 weeks), and the end of study (EOS, varied for each patient). Plasma handling and storage were described previously (7).

Multiplex and ELISA

All biomarkers were measured using the CiraScan multiplex platform (Aushon Biosystems, Inc.), except for BMP-9, Inhibin A, and TGFβ-R3. Inhibin A was quantified using an Inhibin A ELISA assay (Ansh Labs) following the manufacturer's protocols. TGFβ-R3 was tested as previously described (7).

For the BMP-9 ELISA assay, capture antibody (R&D Systems; Catalog # DY 3209) was immobilized onto a bare plate [Meso Scale Discovery (MSD); Catalog # L15XA-3] overnight. Plates were then washed, samples were loaded, and the plates were incubated at room temperature for 2 hours. Detection antibody (R&D systems; Catalog # DY 3209) was applied, and the plates were incubated for 2 hours, followed by the addition of Streptavidin Sulfo-TAG (MSD; Catalog # R32AD-1) and again incubated for 30 minutes. Finally, MSD Read Buffer was added, incubated at room temperature for 10 minutes, and plates were read on the MSD instrument (Sector Imager 2400A).

Statistical analysis

To evaluate biomarker changes on-treatment, L-ratios were calculated using the formula: $\log_2$ (posttreatment level/baseline level) for each analyte, at three time points: C1D8, C2D1, and EOS. Signed-rank tests were used to identify markers that underwent significant modulation upon treatment. Representative markers were graphically illustrated using waterfall plots demonstrating the change from time point 1 to time point 2.

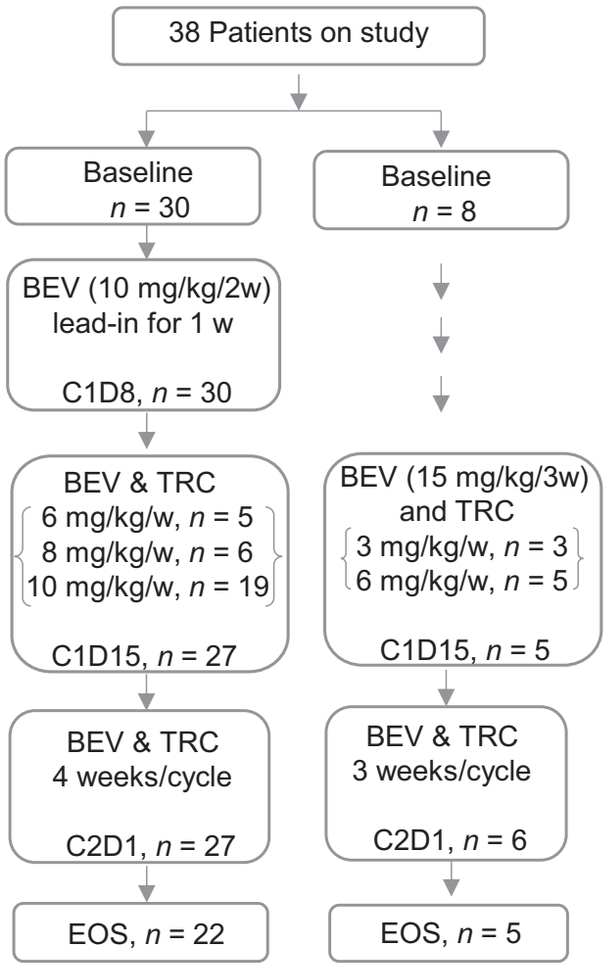

Figure 1.

Consort diagram. The diagram depicts all patients in the parent clinical trial, the patient grouping, the drug schedules, and the number of plasma samples available for biomarker analysis. It is important to note the different doses and schedules for BEV and TRC105 across the individual cohorts.

Spearman rank correlations were calculated for all pairs of biomarkers at baseline, C2D1, and EOS. Hierarchical clustering of all markers at baseline and on-treatment was displayed as dendrograms.

Based upon the response criteria, patients were retrospectively divided into progressive disease (PD) or SD/PR groups. Biomarker differences between these two patient populations were analyzed and illustrated via beeswarm plots to show the baseline variations, as well as the differential modulation of each marker in response to treatment.

Results

Biomarker baseline levels

Thirty-eight cancer patients were enrolled in the phase Ib trial and received escalating doses of TRC105 and one of two fixed doses of BEV (11). Eight patients received both drugs following a 3-week cycle (TRC105 at 3 or 6 mg/kg/week, BEV at 15 mg/kg/3 weeks), whereas 30 patients received a 7-day BEV lead-in

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monotherapy, followed by a 4-week cycle (TRC105 at 6, 8, or 10 mg/kg/week, BEV at 10 mg/kg/2 weeks; Fig. 1). Patient demographics are shown in Supplementary Table S1.

Plasma samples were collected at various time points, but this analysis focused on samples at: (1) baseline, prior to treatment; (2) C1D8, 1 week following treatment with single-agent BEV; (3) C2D1, after one cycle of BEV and TRC105 combination therapy; and (4) EOS. Across all 38 patients who went on study, 27 (71%) EOS samples were available. The most common reason for coming off study was due to disease progression.

In total, 36 biomarkers were evaluated at all 4 time points for each patient. All assays were highly reproducible with coefficients of variation in the range of 5% to 20%. The median and range for each of the biomarkers are shown in Table 1. In this study, 30 patients (79%) had received prior VEGF-targeting therapy, whereas 8 patients (21%) had received no prior anti-VEGF therapy. There were no significant differences in the baseline expression levels of any marker between these two groups of patients. However, we did observe nonsignificant trends that VEGF-A levels were higher ($P = 0.0708$) in patients who had received prior anti-VEGF therapy, whereas IGFBP-2 ($P = 0.0783$) and VEGF-R1 ($P = 0.0822$) were higher in patients who had not received any prior anti-VEGF therapy.

Biomarker modulation upon treatment

To assess drug-induced biomarker modulation, fold change from baseline to C1D8, baseline to C2D1, and C2D1 to EOS was calculated for each patient (Supplementary Table S2). We expanded our interpretation of the current combinatorial trial to include two biomarker studies previously published by our group. TRC105 monotherapy data were derived from a phase I dose-escalation study of patients receiving TRC105 (8); BEV monotherapy data were derived from a phase II study of metastatic colorectal cancer patients receiving BEV and chemotherapy (7), whereas the TRC+BEV combination cohort represents data from the current study (Table 2). Notably, in all three studies, C2D1 samples were available.

BEV-specific effects

In the current trial, we detected a significant increase of PlGF ($P < 0.0001$) at C1D8 after the BEV monotherapy lead-in (Supplementary Table S2). This increase aligns with PlGF increases

Table 1. Levels of biomarkers at baseline and on-treatment

Biomarker	Unit	BL ($n = 37$) Median (range)	C1D8 ($n = 29$) Median (range)	C2D1 ($n = 33$) Median (range)	EOS ($n = 27$) Median (range)
Ang-2	pg/mL	376.6 (201.2–10399.5)	309.8 (157.5–1461.6)	252.9 (148.9–5721.5)	276.5 (0.0–869.2)
BMP-9	pg/mL	184.8 (30.2–331.8)	193.6 (75.5–321.1)	182.0 (54.0–272.9)	161.3 (39.2–280.4)
CRP	μg/mL	4.9 (0.2–2003.5)	3.1 (0.2–1181.6)	6.4 (0.3–1416.4)	8.2 (1.3–525.4)
Endoglin	ng/mL	22.0 (11.4–30.4)	20.8 (13.5–28.4)	99.7 (44.5–177.9)	112.4 (37.0–221.8)
E-Cadherin	ng/mL	9.7 (3.6–35.4)	8.8 (2.1–44.0)	12.3 (5.4–39.4)	10.2 (1.9–40.4)
E-Selectin	ng/mL	45.8 (18.1–253.3)	43.4 (14.7–299.1)	68.7 (34.0–490.5)	76.0 (32.6–327.2)
GRO-α	pg/mL	43.1 (5.5–222.6)	42.9 (11.2–175.9)	56.1 (13.2–227.2)	71.8 (6.2–136.6)
HGF	pg/mL	773.3 (255.8–6572.5)	504.4 (217.7–21410.6)	735.8 (267.2–2758.2)	607.2 (233.6–4090.1)
ICAM-1	ng/mL	447.5 (236.6–2867.8)	419.1 (189.1–1997.9)	442.5 (192.4–2594.2)	512.3 (292.9–1198.9)
IGFBP-1	ng/mL	4.8 (0.3–96.9)	7.5 (0.5–61.1)	5.7 (0.9–228.7)	12.8 (0.4–133.2)
IGFBP-2	ng/mL	874.9 (116.9–4827.6)	879.2 (171.8–2017.2)	963.4 (252.6–3700.8)	987.6 (466.5–5495.7)
IGFBP-3	μg/mL	1.2 (0.3–2.9)	1.4 (0.6–1.9)	1.4 (0.2–2.5)	1.4 (0.2–2.1)
IL6	pg/mL	14.8 (0.9–225.4)	16.0 (2.2–440.9)	15.9 (1.6–348.4)	37.1 (7.9–297.4)
Inhibin A	pg/mL	35.7 (11.1–73.9)	39.6 (23.4–92.0)	38.0 (24.3–62.0)	36.8 (21.5–63.0)
MCP-1	pg/mL	357.9 (108.4–6813.2)	365.4 (104.7–7388.5)	380.9 (118.3–5715.1)	447.9 (141.6–3833.2)
MMP-2	ng/mL	144.5 (52.5–393.0)	146.2 (100.2–254.8)	153.9 (97.2–312.5)	166.4 (117.0–260.0)
MMP-9	ng/mL	297.1 (98.3–1846.6)	213.5 (13.9–1238.2)	345.0 (51.4–1932.9)	245.4 (87.8–1640.7)
OPN	ng/mL	60.2 (9.6–485.8)	62.9 (7.6–127.7)	63.8 (10.7–326.3)	79.2 (26.4–357.1)
PAI-1 active	ng/mL	2.8 (0.0–80.7)	4.9 (0.0–86.8)	5.4 (0.5–95.1)	6.9 (1.1–45.2)
PAI-1 total	ng/mL	8.6 (1.5–68.8)	12.4 (2.1–45.2)	14.7 (1.9–47.2)	15.5 (3.2–105.2)
PDGF-AA	pg/mL	133.4 (9.2–980.1)	122.5 (4.0–1939.5)	197.5 (6.9–1349.2)	231.7 (3.6–1171.9)
PDGF-BB	pg/mL	156.8 (0.2–1564.6)	114.5 (0.4–1918.6)	215.4 (0.8–1810.7)	225.0 (0.7–1140.0)
PEDF	μg/mL	1.5 (0.6–3.0)	1.4 (0.8–2.7)	1.7 (0.2–4.9)	1.5 (0.2–2.6)
PlGF	pg/mL	8.3 (1.2–159.1)	13.3 (7.2–33.3)	16.0 (3.8–210.8)	19.1 (6.0–273.4)
P-Selectin	ng/mL	165.8 (39.2–620.9)	158.6 (38.6–609.6)	227.4 (28.5–955.5)	249.2 (52.3–744.1)
SDF-1	ng/mL	0.9 (0.3–2.1)	0.9 (0.3–3.4)	1.5 (0.5–3.2)	1.6 (0.2–2.8)
TGFβ1	ng/mL	37.8 (12.7–171.0)	33.5 (11.7–203.9)	47.2 (16.0–312.7)	53.3 (26.5–279.5)
TGFβ2	pg/mL	41.8 (11.9–708.7)	34.4 (17.9–707.9)	42.8 (17.3–447.5)	41.3 (20.6–338.2)
TGFβ-R3	ng/mL	83.6 (28.7–178.3)	85.6 (34.8–158.9)	100.2 (45.9–177.6)	99.9 (31.7–180.6)
TSP-2	ng/mL	41.5 (17.6–268.4)	38.5 (15.6–247.4)	39.0 (14.5–193.2)	39.6 (14.7–167.2)
VCAM-1	μg/mL	2.5 (1.2–8.2)	2.4 (1.3–7.8)	3.6 (1.9–8.2)	4.7 (2.5–11.5)
VEGF	pg/mL	99.8 (15.6–506.0)	107.8 (13.5–378.8)	124.6 (27.4–329.7)	137.5 (20.0–303.4)
VEGF-D	pg/mL	812.3 (159.5–1593.5)	858.0 (137.8–1689.4)	777.8 (93.9–1812.6)	971.7 (80.0–1825.0)
VEGF-R1	pg/mL	63.6 (5.7–1734.0)	32.7 (6.2–7205.7)	47.7 (5.5–686.3)	65.1 (18.7–649.9)
VEGF-R2	ng/mL	1.5 (0.5–2.5)	1.6 (0.7–3.2)	1.4 (0.5–2.7)	1.2 (0.3–4.0)
vWF	U/mL	8.6 (1.8–70.8)	7.4 (1.2–67.0)	10.4 (3.7–32.0)	10.2 (3.1–30.1)

Abbreviations: Ang-2, angiopoietin-2; BMP-9, bone morphogenetic protein-9; CRP, C-reactive protein; GRO-α, growth-related oncogene-alpha; HGF, hepatocyte growth factor; ICAM-1, intercellular adhesion molecule-1; IGFBP, insulin-like growth factor binding protein; IL6, interleukin 6; MCP-1, monocyte chemotactic protein-1; MMP, matrix metallopeptidase; OPN, osteopontin; PAI-1, plasminogen activator inhibitor-1; PDGF, platelet-derived growth factor; PEDF, pigment epithelium-derived factor; PlGF, placenta growth factor; SDF-1, stromal cell–derived factor-1; TGF, transforming growth factor; TSP-2, thrombospondin-2; VCAM-1, vascular cell adhesion molecule-1; VEGF, vascular endothelial growth factor; VEGF-R1, VEGF receptor-1; vWF, Von Willebrand factor.

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Table 2. Comparison of biomarker changes from baseline to C2D1 in response to TRC105 monotherapy, BEV monotherapy, and the combination of both TRC105 and BEV

		TRC105 mono		BEV mono		TRC+BEV	
Regimen	**marker**	**Direction**	***P* value**	**Direction**	***P* value**	**Direction**	***P* value**
BEV-specific	PlGF	↓	0.0851	↑	<0.0001	↑	<0.0001
	TSP-2	↑	0.0318	↓	0.0001	↓	0.099
TRC-specific	Endoglin	↑	<0.0001	–	n/a	↑	<0.0001
	E-Cadherin	↑	0.0203	↓	<0.0001	↑	0.103
	E-Selectin	↑	0.0081	↓	<0.0001	↑	<0.0001
	vWF	↑	0.0219	↓	0.0918	↑	0.002
Combo-similar	Ang-2	↓	0.0028	↓	<0.0001	↓	<0.0001
	IL6	↑	0.0255	↑	n.s.	↑	0.006
	VCAM-1	↑	0.0755	↑	<0.0001	↑	<0.0001
Combo-opposing	TGFβ1	↓	0.1485	↓	0.0143	↑	<0.001
	PDGF-AA	↓	0.0147	↓	0.0086	↑	0.002
	PDGF-BB	↓	0.0051	↓	0.003	↑	0.007
	PAI-1 active	↓	0.1491	↓	0.027	↑	0.003
	PAI-1 total	↓	0.0115	↓	0.0002	↑	0.005
Combo-neutralizing	IGFBP-3	↓	0.0025	↑	0.1035	–	n.s.
	VEGF-A	↓	0.1011	↑	0.1392	–	n.s.
	VEGF-D	↓	0.0067	↑	0.0011	–	n.s.

NOTE: Down-pointed arrows indicate an overall decrease of the marker after one cycle of treatment, whereas up-pointed arrows indicate an increase of the marker after one cycle of treatment. *P* values less than 0.05 are considered statistically significant, whereas *P* values between 0.05 and 0.15 represent potential trends. Abbreviations: n/a, not available; n.s., not significant.

after BEV monotherapy treatment for 1 month, shown in Table 2 and in previous reports (7, 12). After addition of TRC105 to BEV, PlGF levels at C2D1 remained significantly higher than baseline levels. In contrast, PlGF was observed to decrease in response to TRC105 monotherapy. These data suggest that increases in PlGF levels in response to TRC+BEV are driven by BEV.

TRC105-specific effects

In this combination study, soluble endoglin (sEng) significantly increased in every patient after addition of TRC105 to BEV as early as C1D15, 1 week after TRC105 was first given (Fig. 2). This increase persisted at C2D1 and EOS ($P < 0.0001$). However, in response to BEV monotherapy, sEng went down in 19 of 29 patients at C1D8 ($P = 0.013$). In addition to sEng, three markers (E-Cadherin, E-Selectin, and vWF) followed the same pattern of change, i.e., increase in response to TRC105 monotherapy, decrease in response to BEV, and increase in response to TRC+BEV (Table 2). These data suggest that in response to TRC+BEV, modulation of sEng, E-Cadherin, E-Selectin, and VWF is mainly driven by TRC105 treatment.

Combination: similar, opposing, and neutralizing effects

Certain biomarkers underwent similar patterns of change regardless of treatment. This was best exemplified by Ang-2, IL6, and VCAM-1. Ang-2 was observed to go down in response to BEV monotherapy, TRC105 monotherapy, and TRC+BEV, whereas IL6 and VCAM-1 were observed to increase in response to BEV monotherapy, TRC105 monotherapy, and TRC+BEV (Table 2). Interestingly, other biomarkers were observed to change in the opposite direction in response to TRC+BEV compared with either monotherapy treatment. Treatment with either TRC105 or BEV monotherapy led to reductions in TGFβ, PDGF-AA, PDGF-BB, PAI-1 active, and PAI-1 total. However, in response to TRC+BEV, all five biomarkers were significantly elevated after one cycle of therapy. Lastly, some drug-specific biomarker changes appeared to be neutralized upon addition of both drugs. In response to TRC105 monotherapy, IGFBP-3, VEGF-A, and VEGF-D decreased, whereas in response to BEV, these markers increased. However, after coadministration of both drugs for 1 month, levels of IGFBP-3, VEGF-A, and VEGF-D were not significantly different from baseline across all patients (Table 2).

Patterns of biomarker change during treatment

Here, we analyzed biomarker changes from baseline to C2D1, as well as from C2D1 to EOS, and characterized two distinct biomarker groups. The first group of markers was characterized by a consistent and continuing increase from baseline to C2D1 and from C2D1 to EOS, such as IGFBP-1, IL6, and PAI-1 total (Fig. 3A–F). The second group of markers also increased from baseline to C2D1; however, it later decreased from C2D1 to EOS such as MMP-9, PEDF, and vWF (Fig. 3G–l). In this study, no biomarker decreased from baseline to C2D1 and then increased from C2D1 to EOS; nor was a consistent decrease observed for any biomarker.

Biomarker profiles in responsive versus nonresponsive groups

In this trial, 2 patients achieved PR, whereas 18 patients achieved SD as best response. We dichotomized the patients into two groups consisting of SD/PR ($n = 20$) and PD groups ($n = 18$). Each marker was tested whether it differed significantly between these two groups at baseline and on-treatment. As shown in Fig. 4A, baseline levels of four markers were significantly lower in the SD/PR group compared with the PD group, including E-Cadherin, HGF, ICAM-1, and TSP-2. At C1D8, no biomarker changes were observed to differ significantly between these two groups. At C2D1, OPN decreased in SD/PR patients but remained stable in PD patients (Fig. 4B). At EOS, TGFβ2 modestly decreased in PD patients, but remained stable in SD/PR patients. Inhibin A, another TGFβ family ligand, decreased in SD/PR patients but increased slightly in PD patients.

Discussion

TRC105 has been studied in patients as a single agent and in combination with VEGF inhibitors (1, 2, 16). In this phase Ib trial, TRC105 was combined with BEV, and more than half of the patients achieved either PR or SD as best response (11), even in

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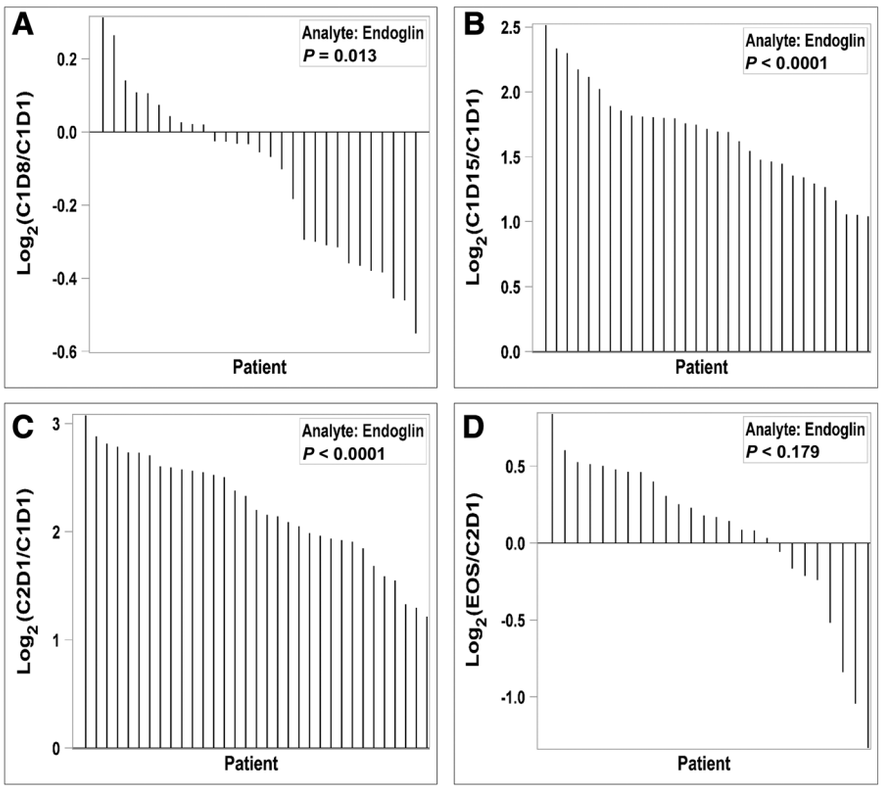

Figure 2.
Levels of sEng increase in patients treated with TRC105. sEng was downregulated from baseline to C1D8 in response to BEV monotherapy (**A**). Upon TRC105 administration, sEng markedly increased in every patient at C1D15 (**B**), C2D1 (**C**), and EOS (**D**).

patients who previously failed anti-VEGF therapy. Plasma samples collected at baseline and during treatment were assessed for various angiogenic and inflammatory protein biomarkers using our validated Angiome multiplex array.

Even using validated approaches, biomarker development is fraught with difficulties, especially in the context of early-stage drug development. Issues around sample size, analytical and clinically validity, replication cohorts, and statistical approaches are all fundamental. Although predictive biomarkers are urgently needed to guide clinical care, early-stage biomarker studies such as this help to refine the operations and methodology, provide biological insights, and lead to novel hypotheses to be formally tested in future studies.

Because we have applied a consistent and standardized approach to evaluate circulating angiogenic biomarkers, we were able to compare the Angiome results from this study to earlier results obtained from single-agent TRC105- or BEV-treated patients. These comparisons may help to elucidate specific biomarker modulations unique to the drug combination compared with either agent alone. There are limitations in making cross-study comparisons, as multiple disease types, different patient populations, and unique drug regimens all can confound interpretation of the results. Although unique biological patterns may provide insights into the differing impact of each agent in patients, these are hypothesis-generating in nature.

In this study, the levels of most biomarkers at baseline were similar to those observed in patients treated with either BEV- or TRC105 monotherapy (7, 8). As discussed, no marker significantly differed between patients exposed to prior VEGF inhibitors and treatment-naïve patients, consistent with the observation that drug-induced biomarker modulation is transient and will return to baseline after treatment with a half-life of around 6 weeks (17). In comparing the three clinical trials described, we established 5 unique groups: BEV-specific, TRC-specific, Combination-similar, Combination-opposing, and Combination-neutralizing.

Drug-specific biomarker changes were observed for both BEV and TRC105. The best example of a BEV-specific change was the increase of PlGF levels, a well-defined pharmacodynamic biomarker for both small-molecule VEGFR-2 inhibitors and monoclonal antibodies targeting soluble VEGF (17–19). The function of increased circulating PlGF is not fully understood, but it likely reflects the activation of compensatory angiogenic pathways upon the blockage of VEGF signaling (17). The best example of a TRC105-specific change was the increase of sEng, first reported by our group (8). We detected robust increases of sEng in response to TRC105 at all time points tested, including the C1D15 time point which was only 1 week after TRC105 administration (Fig. 2). As presented, the increases in sEng persisted throughout the continuum of TRC105 treatment in all patients. In contrast, BEV monotherapy led to decreased levels of sEng. The downregulation of sEng in response to 1 week of BEV treatment was confirmed recently in our Angiome analysis of a phase Ib trial where TRC105 was combined with pazopanib in patients with advanced soft-tissue sarcoma (NCT01975519). Patients received pazopanib lead-in monotherapy for 4 weeks prior to TRC105 treatment, and sEng was significantly down regulated ($P = 0.012$; ref. 10). When both drugs were present, TRC105 clearly exerts a dominant effect on changes observed in sEng. sEng is an actively studied

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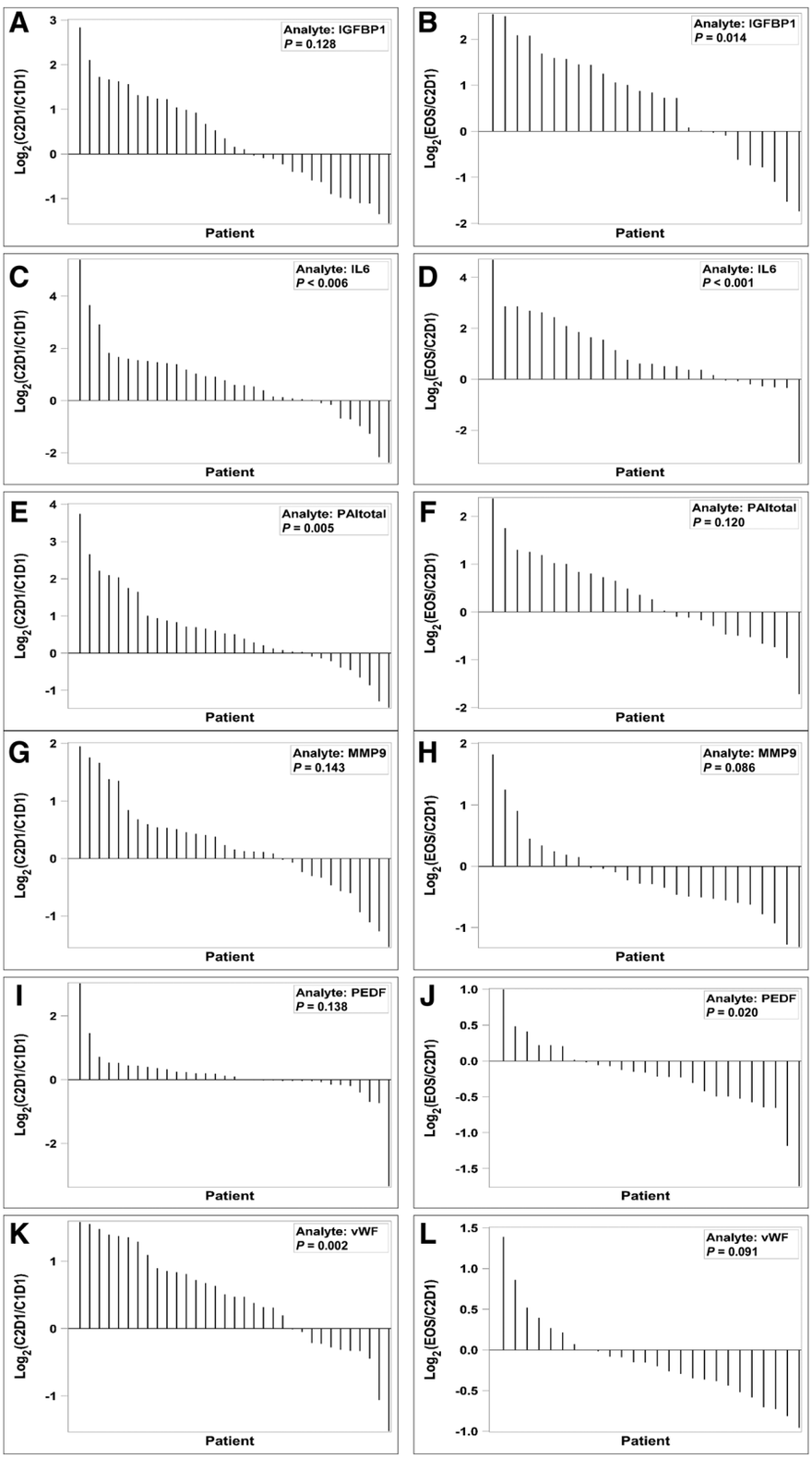

Figure 3.

Distinct pharmacodynamic patterns emerge during treatment. The change in biomarker levels during treatment was examined at early times in the setting of response (baseline to C2D1) and at later times in the setting of progression (C2D1 to EOS). **A–F,** Biomarkers in this group demonstrated a consistent increase at both early times from baseline to C2D1 (**A**, **C,** and **E**) as well as at later times from C2D1 to EOS (**B**, **D**, and **F**). **G–I,** Biomarkers in this group demonstrated initial increases at early times from baseline to C2D1 (**G**, **I**, and **K**), but decreased during treatment from C2D1 to EOS (**H, J**, and **I**).

biomarker, and it has been implicated in various pathologic conditions, including hereditary hemorrhagic telangiectasia (20), preeclampsia (21), atherosclerosis (22), and cancer (2). Locally increased sEng levels are capable of scavenging ligands such as TGFβ and BMPs, affecting the angiogenic potential of tumor-associated endothelial cells (23). However, the exact role

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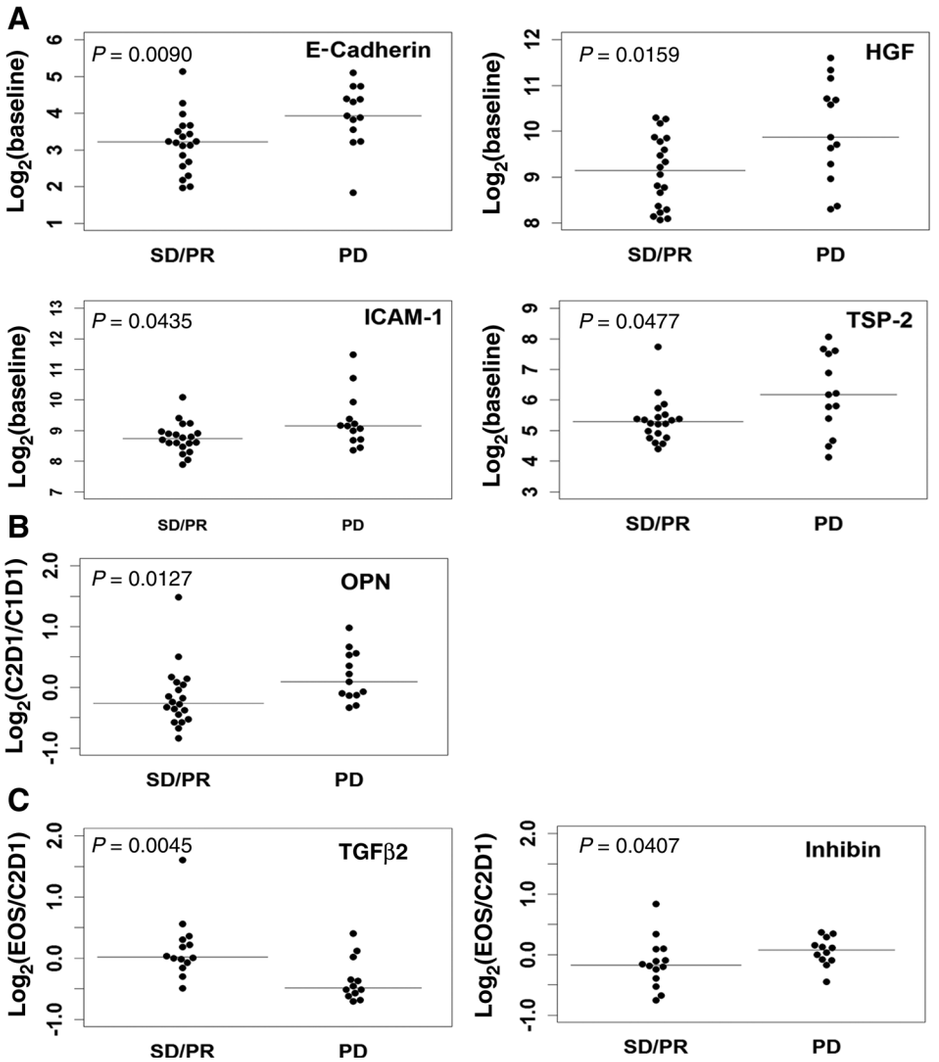

Figure 4.
Biomarkers associated with clinical response. Markers were tested for association with patients categorized into two groups representing SD/PR and PD groups. **A,** Baseline levels of E-Cadherin, HGF, ICAM-1, and TSP-2 were observed to be significantly lower in SD/PR patients compared with patients exhibiting PD. **B,** OPN levels decreased from baseline to C2D1 in SD/PR patients, whereas OPN levels slightly increased from baseline to C2D1 in PD patients. **C,** TGFβ2 levels remained stable in SD/PR patients from C2D1 to EOS and decreased in PD patients from C2D1 to EOS. Inhibin levels decreased in SD/PR patients from C2D1 to EOS and increased in PD patients from C2D1 to EOS.

of sEng in mediating sensitivity or resistance to either TRC105 or BEV remains unclear.

We noted that when biomarkers exhibited similar patterns of change in response to BEV monotherapy and TRC105 monotherapy, the combination of both drugs often also led to the same pattern. This was observed for Ang-2, where levels decreased in response to all three therapeutic interventions (BEV, TRC105, and combination); and IL6 and VCAM-1, where levels increased in response to these treatments. However, for several markers, we observed a surprising opposing effect, i.e., the effect on biomarkers caused by the combination of both drugs was the opposite of that caused by either drug alone. Several markers that decreased in response to either drug, significantly increased upon drug coadministration, included TGFβ1, PDGF-AA, PDGF-BB, and PAI-1 (total and active). TGFβ1 is known to be a strong inducer of tumor cell proliferation, angiogenesis, and metastasis, and induction of TGFβ1 often correlates with poor prognosis (24). TGFβ1 has both tumor-inhibitory and -promoting roles in context-dependent manners (24). PDGF family members mediate a variety of biological responses, such as proliferation, chemotaxis of smooth muscle cells, and fibroblasts (25), and PDGF signaling is required for the recruitment of stromal fibroblasts for tumor angiogenesis and growth (26, 27). PAI-1 is a key regulator of extracellular matrix remodeling, and its overexpression has been associated with increased tumorigenicity in multiple tumor types (28–31). It should be noted that these proteins are intimately related as both PDGFs and PAI-1 are positively regulated by TGFβ1 (32). Interestingly, strong clustering of these markers was also observed in dendrograms provided in the Supplementary Data (Supplementary Fig. S1A–S1D). The impact of TGFβ1 upregulation in this trial, as well as multiple downstream effectors, needs to be further interrogated in larger, more controlled clinical studies.

In addition to the combination-similar and combination-opposing effects, we were also able to detect neutralizing effects when combining TRC105 and BEV. This was observed for VEGF-A, VEGF-D, and IGFBP-3; all three markers increased in response to BEV monotherapy, yet decreased in response to TRC105 monotherapy. These opposing effects appeared to offset each other in patients treated with both drugs, resulting in no overall change in VEGF-A, VEGF-D, and IGFBP-3 after treatment with BEV

and TRC105 for one cycle of therapy. How such neutralizing effects can contribute to overall drug efficacy or resistance requires further investigation.

Lastly, in this trial, there were 2 patients who achieved PR and 18 patients who achieved SD of up to 6 months or longer. To test if unique biomarker features were associated with clinical outcome, we retrospectively divided patients into SD/PR (responsive) and PD (nonresponsive) groups and compared baseline biomarker expression as well as on-treatment changes (Fig. 4). SD/PR patients had low baseline levels of E-Cadherin, HGF, ICAM-1, and TSP-2. With the exception of HGF, these markers play pivotal roles in extracellular matrix remodeling, degradation, migration, and metastasis (33). On treatment, the SD/PR group showed reduction of OPN at C2D1, of inhibin at EOS, and stable TGFβ2 levels at EOS. The possible predictive value of these markers needs to be validated in larger, randomized trials.

In conclusion, the combination of BEV and TRC105 showed encouraging signs of clinical efficacy. Importantly, unique biomarker modulations were observed in patients treated with the combination of BEV and TRC105, distinct from those observed in patients treated with either single agent. Because TRC105 is being tested with multiple anti-VEGF agents across a variety of indications, potential biomarker differences should be further explored to better understand drug efficacy and related resistance mechanisms. Our consistent application of the Angiome in clinical trials exploring the combination of TRC105 with other drugs will undoubtedly deepen our understanding of the mechanisms involved in delayed drug resistance and potentially help identifying novel targets to improve drug efficacy.

Disclosure of Potential Conflicts of Interest

B. Adams is Sr. VP Clinical Operations at, and has an ownership interest (including stock, patents, etc.) in, TRACON Pharmaceuticals, Inc. D. Alvarez is Director of Clinical Operations at, and has an ownership interest (including stock, patents, etc.) in, TRACON Pharmaceuticals, Inc. C.P. Theuer has an ownership interest (including stock, patents, etc.) in TRACON Pharmaceuticals, Inc. H.I. Hurwitz is an employee at Genetech/Roche, has an ownership interest (including stock, patents, etc.) in Roche, and is a consultant/advisory board member for Genentech/Roche. A.B. Nixon reports receiving commercial research grant from Tracon Pharma, Genentech, Novartis, Acceleron Pharma, and MedPacto Pharma, and is a consultant/advisory board member for Pfizer and Eli Lilly. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Liu, M.D. Starr, C. Rushing, H. Pang, H.I. Hurwitz, A.B. Nixon
Writing, review, and/or revision of the manuscript: Y. Liu, C. Rushing, H. Pang, C.P. Theuer, H.I. Hurwitz, A.B. Nixon
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Molecular Cancer Therapeutics

Modulation of Circulating Protein Biomarkers in Cancer Patients Receiving Bevacizumab and the Anti-Endoglin Antibody, TRC105

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