Biomarker Modulation in Patients Receiving TRC105 and Bevacizumab in a Phase Ib Clinical Trial

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Introduction

Endoglin is a receptor expressed on proliferating endothelial cells which modulates angiogenesis through TGF-β and BMP signaling[1]. Endoglin expression is associated with poor prognosis in many malignancies, including colorectal cancer. Furthermore, endoglin is implicated as a mechanism of anti-angiogenic resistance, as genetic knockdown of endoglin sensitizes tumors to VEGF inhibition[2]. TRC105 is an IgG1 monoclonal antibody that binds endoglin with high avidity, competitively inhibits BMP binding, and disrupts angiogenesis *in vitro* and *in vivo*[3]. TRC105 has been evaluated in multiple phase I clinical trials as a single agent and in combination with inhibitors of the VEGF pathway. A phase Ib/2 trial of patients given bevacizumab (BEV) with TRC105 indicated the combination was safe and efficacious in patients who progressed on prior BEV therapy[4]. Using multiplex ELISA based analysis, we herein characterize alterations in the circulating angiome and TGFβ-related biomarkers of patients treated with combination of BEV and TRC105.

Methods

Biomarker analyses were performed on 38 advanced refractory cancer patients who progressed on prior BEV-containing regimens. Plasma from patients treated with escalating doses of TRC105 (3-10 mg/kg/weekly) and two doses of bevacizumab (15 mg/kg/3 weeks or 10 mg/kg/2 weeks) was sampled at several time points [baseline, C1D8, C1D15, C2D1, (C=cycle, D=day)] and at the end of study (EOS). Thirty patients (80%) received BEV as a lead-in monotherapy for 1 week. Thirty six biomarkers related to angiogenesis, tumor growth, vascular activation and inflammation were assayed using the Aushon SearchLight multiplex system, as shown in Table 1.

Patient Characteristics

Age	Median (range)	63.5 (44-83)	
Sex	Male, n (%)	15	(39%)
	Female	23	(61%)
Race	Caucasian	30	(79%)
	Other	8	(21%)
Tumor	Colon/Colorectal	15	(39%)
Type	Ovarian	11	(29%)
	Others	12	(32%)

Table 1. List of biomarkers evaluated.

Soluble angiogenic biomarkers		TGFβ-related makrers	Vascular activation and inflammation biomarkers	
Ang-2	PDGF-AA	BMP-9	CRP	PAI-1 Active
HGF	PDGF-BB	Endoglin	E-Cadherin	PAI-1 Total
IGFBP-1	PlGF	Inhibin A	E-Selectin	P-Selectin
IGFBP-2	TSP-2	OPN	Gro-α	SDF-1
IGFBP-3	VEGF-A	TGF-β1	ICAM-1	VCAM-1
MMP-2	VEGF-D	TGF-β2	IL-6	vWF
MMP-9	sVEGF-R1	TGFβ-R3	MCP-1	
PEDF	sVEGF-R2			

Results

❖ Patient were enrolled on this study and plasma samples were collected throughout the duration of treatment. The statistical analyses presented focus on two time points: baseline, C2D1, and EOS.

❖C2D1 was assessed to reflect treatment-related changes after 4 weeks of treatment. C2D1 samples were available from 33 patients. Wilcoxon signed rank tests reveal that multiple angiogenic factors were significantly up regulated at C2D1 (Table 2). Representative Waterfall plots were shown for selected significant biomarkers of change (Figure 1).

❖ EOS levels were assessed to reflect potential changes associated with disease progression. EOS samples were available from 27 patients. Wilcoxon tests revealed significantly modulated markers by EOS (Table 2), as shown in Waterfall plots (Figure 2) .

❖ Spearman rank test identified statistically significant pair-wise correlations, e.g., TGF-β1 and PDGF-AA, PDGF-AA and PDGF-BB, PAI-1 active and PAI-1 total. All correlations are positive (data not shown).

Table 2. Significantly modulated biomarkers at C2D1 and EOS.

Baseline to C2D1				C2D1 to EOS	
Marker	**p-value**	**Marker**	**p-value**	**Marker**	**p-value**
Biomarkers significantly increase in response to TRC105 and BEV					
Endoglin	<0.0001	vWF	0.002	VCAM-1	<0.0001
E-Selectin	<0.0001	PAI-1 active	0.003	IL-6	<0.001
PlGF	<0.0001	CRP	0.004	ICAM-1	0.001
SDF-1	<0.0001	PAI-1 total	0.005	PlGF	0.002
VCAM-1	<0.0001	IL-6	0.006	VEGF-R1	0.009
TGF-β1	<0.001	PDGF-BB	0.007	IGFBP-1	0.014
P-Selectin	0.001	Inhibin A	0.026	OPN	0.016
PDGF-AA	0.002			TGFβ-R3	0.023
Biomarkers significantly decrease in response to TRC105 and BEV					
Ang-2	<0.0001			IGFBP-3	0.014
BMP-9	0.011			PEDF	0.02
				BMP-9	0.027

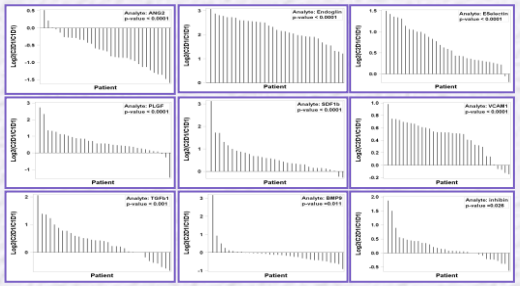

Figure 1. Waterfall plots show significantly modulated markers from baseline to C2D1. Top six, most significant markers with $p<0.0001$. Bottom three, TGF-β related markers with $p<0.05$.

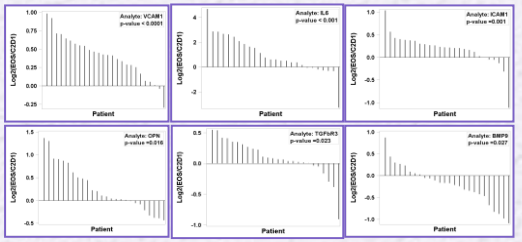

Figure 2. Waterfall plots show significantly modulated markers from C2D1 to EOS. Top three, most significant markers with $p<0.001$. Bottom three, TGF-β related markers with $p<0.05$.

❖ Of the 33 patients with response data available, 6 patients achieved partial response (PR) and 14 patients had stable disease (SD). These 20 patients were lumped as *responders*. The remaining 13 patients progressed on study and were considered *non-responders*. A dichotomized rank sum test was performed to identify differentially regulated biomarkers between responders (SD/PR) and non-responders (progressive disease, PD) groups (Figure 3). Panel A reflects the difference in baseline levels of each marker while Panel B reflects the differential fold-change from baseline to C2D1 in responder versus non-responder groups.

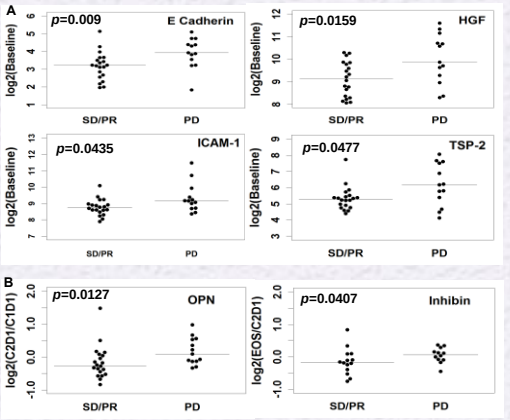

Figure 3. Beeswarm plots show differentially regulated markers at baseline(A) and C2D1(B).

❖ We have now assessed multiple clinical studies with TRC105 and BEV, either alone or in combination, and noted differential biomarker modulation across these studies. To facilitate comparison, a list of markers across three clinical studies is shown in Table 3. *TRC105 mono* represents the evaluation of biomarkers in 32 cancer who received TRC105 monotherapy[5]. *BEV mono* represents the evaluation of 38 colon cancer patients received BEV and supportive chemotherapy[6]. *TRC105 +BEV* represents the current study of 38 patients who received both drugs. In Table 3, significant changes from baseline to C2D1 were shown along with fold changes (FC) from baseline and corresponding p-values. N.S. - Not significant

Table 3. Comparison of biomarker modulation at C2D1 across studies.

	TRC105 mono[5]		BEV mono[6]		TRC105+BEV	
	FC	p-value	FC	p-value	FC	p-value
Decreases with TRC105 mono, BEV mono, and combination						
Ang-2	0.91	0.0028	0.77	<0.0001	0.82	<0.0001
Decreases with TRC105 mono, increases with BEV mono, and combination						
PlGF	1.04	N.S.	1.98	<0.0001	1.49	<0.0001
Increases with TRC105 mono and combination, decreases with BEV mono						
CRP	1.46	N.S.	0.36	0.0356	3.13	0.004
Endoglin	2.70	<0.0001	0.94	0.005	3.31	<0.0001
E-Selectin	1.33	0.0081	0.82	<0.0001	1.41	<0.0001
IL-6	1.48	0.0255	0.83	N.S.	3.30	0.006
vWF	1.38	0.0219	0.98	N.S.	1.41	0.002
Decreases with TRC105 mono and BEV mono, increases with combination						
P-Selectin	1.14	N.S.	0.86	0.0061	1.24	0.001
PAI-1 active	1.18	N.S.	0.72	0.027	2.89	0.003
PAI-1 total	0.89	0.0115	0.66	0.0002	1.86	0.005
PDGF-AA	0.93	0.0147	0.67	0.0086	2.28	0.002
PDGF-BB	1.10	0.0051	0.66	0.003	2.02	0.007
TGF-β1	1.05	N.S.	0.81	0.0143	1.46	<0.001
Increases with TRC105 mono, BEV mono, and combination treatment						
VCAM-1	1.12	N.S.	1.32	<0.0001	1.29	<0.0001

Conclusions

❖ This report represents the first circulating biomarker profile from cancer patients treated with TRC105 and BEV. Unique biomarker modulations were observed when compared to changes observed in patients receiving either drug alone. Five distinct Pharmacodynamic patterns were observed (Table 4).

❖ BMP-9, the selective ligand for endoglin[7], was evaluated for the first time in cancer patients treated with TRC105 and BEV. BMP-9 levels significantly decreased upon TRC105 and BEV administration.

❖ Biomarkers were differentially regulated between responders and non-responders; these findings suggest that some patients may exhibit increased sensitivity to the combination regimen of TRC105 and BEV.

References

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